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EFFECTIVE DURATION OF SOME AGENTS USED FOR DENTIN STERILIZATION[1]

SAMUEL SELTZER, D.D.S.[2]

School of Dentistry, University of Pennsylvania and Children's Dental Clinic, Philadelphia Mouth Hygiene Association, Philadelphia, Pa.

As far back as 1840, when the use of silver nitrate was first reported in dentistry (5), it seemed that some sort of chemical application to a carious cavity was desirable. With the advent of Miller's chemicoparasitic theory of tooth decay, it became logical to assume that, since bacteria are at least invariably associated with dental caries, some effort should be made to eliminate them or control their growth in the cavity. Toward this end, many germicides and antiseptics have been advocated. Many have been discarded in favor of newer and more powerful drugs. A few, of which silver nitrate and phenol are notable examples, have been in favor through the years and are still in widespread use. The employment of cavity sterilization, however, is not universal. Many dentists do not subscribe to routine application of antiseptics to dentin, prior to the insertion of filling material. While in some cases this may be attributed to negligence, in others, it results from doubt concerning the efficacy of such treatment.

The reasons given by those who doubt the need for cavity sterilization may be listed as follows: 1. Insertion of a filling which hermetically seals the dentin from further penetration of saliva is sufficient insurance against recurrence of caries. 2. Drugs which are powerful enough to kill all the bacteria in the dentin are probably injurious to the pulp. 3. In deep cavities the pulp is probably already infected and it is only a question of time before root-canal therapy will be necessary.

The author has attempted to compare the efficiency of various medicaments used in cavity sterilization by *in vivo* experimentation (2). At first it appeared that some drugs were better than others for this purpose. The order of efficiency was as follows: 1. Morson's "Kreosote"; 2. 10 per cent aqueous solution of iodin;[3] 3. Pure phenol; 4. Physiologic sodium chlorid solution; 5. Isotonic solution of iodin;[4] 6. 50 per cent solution of thymol in 95 per cent ethyl alcohol; 7. 50 per cent solution of phenol in 95 per cent ethyl alcohol; 8. Howe's ammoniacal silver nitrate; 9. 95 per cent alcohol.

[1] This study was aided by a grant from the William John Gies Fund of the American College of Dentists. Received for publication December 12, 1941.

[2] Now on active duty with the U. S. Army (D. C.).

[3]

Iodin	10 gms.
Pot. Iodid	20 gms.
Water	100 cc.

[4]

Iodin	1.58 gms.
Pot. Iodid	2.53 gms.
Water	100 cc.

It was desirable to check again the bacteriologic status of the dentin of those cavities upon which the work was done. It was felt that this would be of value in answering several questions. What effect does sealing have on the bacteria left in the dentin? What effect does time have on the action of medicaments used? Does the bacteriologic status of the dentin remain the same? Therefore, the cavities were not filled with cement and amalgam in the usual manner. Instead, an intervening layer of steril base plate gutta percha was placed between the amalgam and the underlying dentin. This procedure eliminated any antibacterial influence of cement or amalgam on the dentin at the floor of the cavity. Whenever possible, a ledge of sound dentin, upon which the amalgam could rest, was provided (*fig. 1*). The gutta percha was used for several reasons: 1. It is inert and has no influence on the underlying dentin from a bacteriologic standpoint; 2. It is easily sterilized in the open flame; 3. It can be removed easily.

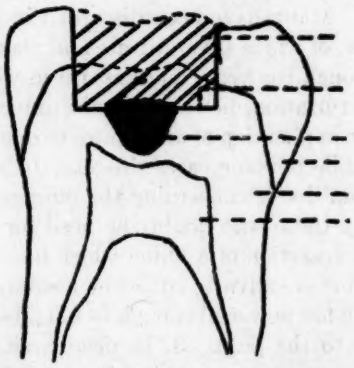

FIG. 1. Illustration of method by which cavity was sealed

The patients were not seen again for 1 year. When the patients were recalled, the teeth that had been treated previously were painted with tincture of iodin followed by alcohol and chloroform. The amalgam was ground out with steril burs exposing the underlying gutta percha. The latter was removed in one piece with a steril excavator. Shavings of the dentin, made with a steril bur, were then immediately inoculated into a tube of Rosenow's brain broth.[5] The tooth was tested for vitality, refilled and the patient was dismissed.

About 300 cavities were originally treated but only 93 (31 per cent) returned for this check-up 1 year later. Although the amount of data presented is small, it was felt that it would be better to make a report now, rather than wait until more evidence is collected. A collection of cases in numbers sufficient to be authoritative would probably take several years. It is hoped, in the meantime, that this paper will stimulate further research along these lines so that a

[5] This medium is excellent for the growth of streptococci and had already been tested previously for growth of lactobacilli with favorable results.

clear perception of the problem of cavity sterilization will emerge in a shorter time.

RESULTS

When 3 consecutive cultures of the dentin are made, 8 possible combinations of results may be obtained. The results of the 3 cultures are listed in consecutive order at the top of columns 4 to 7, 9 to 12, inclusive in Table I. The first sign refers to the result of the original culture which was taken after the cavity was prepared but before any medicament was applied to the dentin. The second sign

TABLE I

1	2	3	4	5	6	7	8	9	10	11	12	13
GROUP	NO. OF CASES	TREATMENT	SEQUENCES OF CULTURES									
			+++	0++	00+	+0+	Total	000	+00	++0	0+0	Total
A	12	No sterilization	3	3	2	1	9	2	1	0	0	3
B	8	Physiologic sodium chlorid sol'n.	3	0	0	3	6	1	1	0	0	2
C	4	95 per cent ethyl alcohol	4	0	0	0	4	0	0	0	0	0
D	6	Isotonic solution of iodin	2	0	0	0	2	2	1	0	1	4
E	6	10 per cent aqueous sol'n. of iodin	1	1	0	4	6	0	0	0	0	0
F	10	50 per cent solution of thymol in alcohol	4	0	0	2	6	0	1	3	0	4
G	9	50 per cent solution of phenol in alcohol	5	1	0	2	8	0	0	0	1	1
H	14	Pure phenol	1	1	1	8	11	2	0	1	0	3
I	9	Howe's ammoniacal silver nitrate	6	0	0	0	6	1	0	2	0	3
J	15	Morson's "kreosote"	3	2	0	0	5	2	3	5	0	10
	93		32	8	3	20	63	10	7	11	2	30

Growth, +; No growth, 0.

refers to the result of the second culture which was taken 1 to 2 weeks after the medicament had been applied to the dentin. The third sign refers to the result of the culture taken 1 year later, the procedure for which is described in the present report. Thus, reading from left to right, + + + means that all 3 cultures were positive, whereas 0 + + means that the first culture was negative but the second and third cultures were positive, etc. In the middle of the table is a triple line. To the left of this line have been placed all sequences of the 3 cultures which end positively; to the right, all which end negatively.

From the data in Table I, it is possible to institute comparisons between the initial culture and the second culture, the second culture and the third culture, the initial culture and the third culture.

The first group of comparisons has already been reported (2). The second group of comparisons would not reveal a true picture because when selected cavities were prepared and the dentin was cultured and treated with an antiseptic, the cavities were sealed with gutta percha and a quick-setting zinc-oxid and eugenol cement. One to two weeks later, the patients were brought back so that the dentin could be cultured again and in a few cases, some of this temporary filling broke out between visits. The dentin was cultured anyway, but the results of these doubtful cases were not incorporated into the final report (2). However, the dentin of these cases was again treated with an antiseptic and the cavity was sealed in the same manner as has already been described in this paper (with gutta percha and amalgam). Some of these cases returned 1 year later and were cultured. It would, therefore, be unfair to compare the second culture and the third culture since some of the positives of the second culture were due directly to contamination.

Making the third group of comparisons, however, permits an evaluation of the effectiveness of the treatment since it compares the results of cultures of the dentin taken before and 1 year after antiseptic treatment and sealing. The following form will simplify analysis of the data of Table I:

INITIAL CULTURE	CULTURE AFTER 1 YEAR	
	+	0
+	Ineffectual	Effectual
0	Discard	Casts no light on efficacy of treatment

In comparing the initial culture and the third culture there are 4 possible combinations of results: 1. If both the initial culture and the culture taken after 1 year turned out positive, it was decided to designate the treatment in question as "ineffectual." This designation is obvious since no change in the status of the dentin was brought about. 2. If the initial culture was positive and the culture taken after 1 year was negative, it was decided to designate the treatment in question as "effectual" since the dentin was rendered steril within 1 year after treatment. 3. If the initial culture was negative and the culture taken after 1 year was positive, it was decided to discard these results as "without significance as to efficacy."[6] 4. If the initial culture was negative and the

[6] The reasons for this will become more apparent when the following form is examined:

INITIAL CULTURE	1 YEAR CULTURE	
	True + (i.e. +)	False + (i.e. 0)
False 0 (i.e. +)	Ineffectual (a)	Effectual (b)
True 0 (i.e. 0)	Contamination (c)	Without significance (d)

A. If the negative obtained on the initial culture was a false negative (i.e. actually posi-

culture taken after 1 year was negative, it was decided to eliminate those cases as "casting no light on the efficacy of the treatment."

Applying this method of analysis to the results after Treatment A, the following is obtained:

INITIAL CULTURE	CULTURE AFTER 1 YEAR	
	+	0
+	Ineffectual 4	Effectual 1
0	Discarded 5	Casts no light on efficacy of treatment 2

When all treatments are recorded in this manner, the total results may be compared in Table II.

Thus, in approximately 56 per cent of all cases the treatment was "ineffectual," in approximately 19 per cent treatment was "effectual" and approximately 25 per cent of the cases were "without significance as to efficacy." The column headed "per cent effectual" in Table II is inserted merely for convenience. It is realized that the number of observations is too small to do more than to suggest along which lines further study might be profitable.

In 9 of the 10 types of treatment attempted for the dentin of prepared cavities (A to I incl.) the treatment was predominately "ineffectual" (*fig. 2*). The last treatment (J) was the only one in which more cases were placed in the "effectual" category than in the "ineffectual."

The presence of bacteria in the dentin does not necessarily mean the presence of decay. However, the fact that bacteria which are commonly associated with dental caries can continue to live in the dentin after the latter has been treated with an antiseptic and sealed from the saliva must result in regarding the antiseptic with suspicion. Until evidence is offered showing these bacteria to

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tive) and the positive obtained on the 1 year culture was a true positive, then the treatment should be classified as "ineffectual" (a).

B. If the negative obtained on the initial culture was a false negative (i.e. actually positive) and the positive obtained on the 1 year culture was a false positive (i.e. actually negative), then the treatment should be classified as "effectual" (b).

C. If the negative obtained on the initial culture was a true negative and the positive obtained on the 1 year culture was a true positive then the treatment should be discarded as due to "contamination". (c).

D. If the negative obtained on the initial culture was a true negative and the positive obtained on the 1 year culture was a false positive (i.e. actually negative), then the treatment should be discarded as "without significance as to efficacy."

The treatments yielding this combination of results (initial culture negative, culture after 1 year positive) certainly should not be called "effectual" (b). If they were ruled as "contamination" (c) or "without significance" (d), they would be discarded. Since there are 2 chances (c and d) that they would be discarded against one chance (a) that they would be utilizable in the calculations, there is justification for discarding them.

be harmless inhabitants of the dentin, the burden of proof as to the inocuousness of these organisms must rest upon those who belittle the need for cavity sterilization. Under any circumstances, however, in view of the overwhelming majority of cases in which attempts at sterilization of the dentin were unsuccessful, it seems desirable to continue the search for a method of accomplishing sterility for the following reasons: 1. We depend upon clinical signs to tell us when all caries is removed. We stop excavating when all softened and discolored tissue is gone and when the underlying dentin feels hard to the explorer. Prime (3), with the use of silver nitrate, has pointed out how inadequate this method is. "It is necessary to use Howe's solution on only a few cases to learn how poor our methods are. Indeed, in some cases, a wall may have caries as much as a millimeter thick on it, and we would not, with our old methods know that it was there." 2. There is histologic proof that bacteria may be left in the dentin after

TABLE II

1	2	3	4	5	6
GROUP	NUMBER OF CASES	TREATMENT EFFECTUAL	PER CENT EFF.	TREATMENT INEFFECTUAL	W/O SIGNIFICANCE—
A	12	1	20	4	7
B	8	1	14	6	1
C	4	0	0	4	0
D	6	1	33	2	3
E	6	0	0	5	1
F	10	4	40	6	0
G	9	0	0	7	2
H	14	1	10	9	4
I	9	2	25	6	1
J	15	8	73	3	4
Total.....	93	18	19	52	23

cavity preparation. Zander (4) has shown that in 4 of 10 teeth sectioned after cavity preparation, microscopic examination has revealed bacteria in the dentinal tubules. 3. There is bacteriologic proof that bacteria may be left in the dentin after cavity preparation. Seltzer (1) grouped all cavities into 3 divisions; shallow, medium, and deep. After cavity preparation, shavings from the dentin were cultured in all cases. Bacterial growth was shown from 41.6 per cent of the shallow cavities, from 84.0 per cent of the medium cavities and from 93.6 per cent of the deep cavities.

The question might be asked, "Even if living bacteria may still be demonstrated in the dentin after all decay has been removed, what difference does that make? As long as a good restoration, which hermetically seals the dentin against further penetration of saliva, is inserted there is nothing more to worry about." In answer to this it may be pointed out that no evidence exists to show that the process of dental caries will not progress if the dentin is merely properly sealed. There is evidence, however, to show that sealing alone can not

be depended upon to sterilize the dentin while it is possible that our inability to sterilize the dentin is the predominate reason for recurrence of decay under old restorations.

Examining the data in Table I reveals several facts: 1. Of 70 cases that were positive at the first culturing, 27 (39 per cent) became negative at the second culturing. 2. Of 70 cases that were positive at the first culturing, 18 (26 per cent) became negative at the third culturing. Thus, despite sealing, fewer cases were negative at the end of 1 year than at the end of 2 weeks.

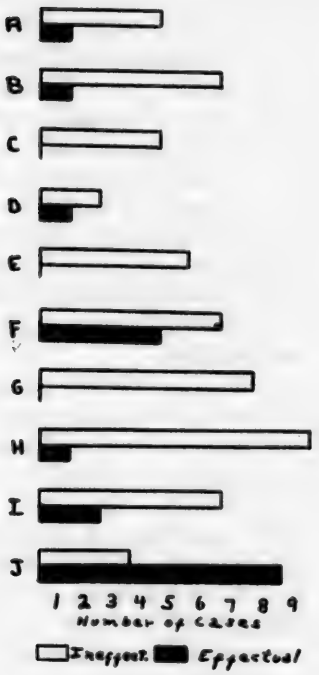

FIG. 2

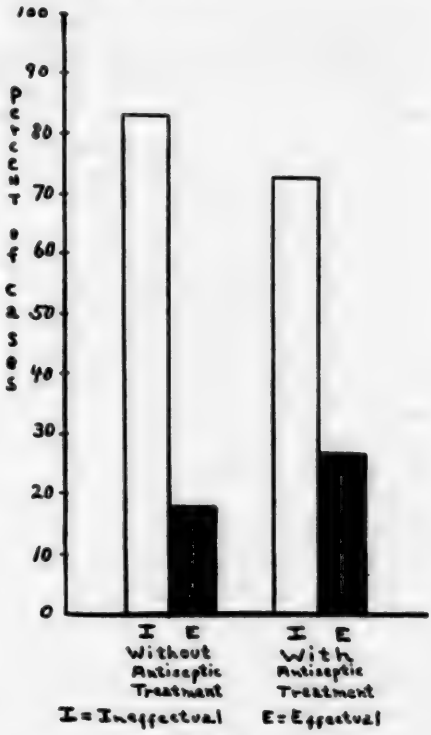

FIG. 3

FIG. 2. Efficacy of treatment with various antiseptics
FIG. 3. Comparison of results with and without treatment

In examining the data in Table II, several other facts are obvious: 1. (a) In control group A, 4 of 5 cases remained positive 1 year later despite sealing. (b) In the sodium chlorid group B, 6 of 7 cases remained positive 1 year later despite sealing. Thus, in all, 10 (83 per cent) of 12 cases were positive after 1 year where sealing alone was depended upon to sterilize the dentin. 2. In 42 (73 per cent) of 58 cases (groups C to J incl.) sealing with a filling plus antiseptic treatment failed to change an initially positive culture into a negative culture, i.e., failed to sterilize the dentin (*fig. 3*).

It is obvious that merely sealing a cavity is not an effective way of sterilizing the dentin. It might be argued that amalgam does not hermetically seal a cavity, that there are microscopic spaces between the wall of the cavity and the filling through which contamination could take place. In other words the question arises, "Can positive cultures, obtained 1 year after treatment, indicate leakage?" Examination of the data of Table I reveals that of 23 cases that were originally negative (total of columns 5, 6, 9, and 12), 12 (more than 50 per cent) remained negative 1 year later. Obtaining a positive culture in the 11 other cases could be due to 2 factors: 1. An error in bacteriologic technic or 2. Leakage. The results point to the probability that in 8 of these 11 cases, the initial negative was an error in technique since both of the subsequent cultures were positive. It seems, therefore, that in only 3 cases (13 per cent) can the final positive be attributable directly to leakage. It is noteworthy to recall that the difference between the percentage of initially positive cases that were negative at the second culturing (39 per cent) and the percentage of initially positive cases that were negative at the third culturing (26 per cent) is 13 per cent. This last figure seems to be a fair index of the amount of leakage that has occurred.

A point that should now be emphasized is, that if some positives were due to leakage, the number of cases effectively treated by antiseptics is at least 13 per cent greater than *fig. 3* indicates. This is sufficient reason for continuation of the search for a method of sterilization of the dentin in a greater percentage of cases. Accomplishment of sterility of the dentin is desirable, even though the exact value of such attainment has not yet been established.

The reasons propounded by those opposed to the practice of cavity sterilization might be discussed briefly, now. 1. Hermetic seal prevents recurrence of decay. This has already been discussed. It has been pointed out that no evidence exists to support this contention. The evidence presented in this paper shows that a hermetic seal will not sterilize the dentin. Whether or not the bacteria remaining can cause decay has yet to be proven. 2. Drugs injurious to the pulp. It is true, in certain cases where caustics have been used, that the pulp may be injured. However, the trend is away from use of these njurious drugs in dentistry. Of the group of drugs tested by the author, none injured the pulp 1 year after use (according to the pulp test) regardless of the idepth of the cavity when the medicament was applied. 3. Pulp already infected in borderline cases. Although this opinion is held by some men, there is little evidence in favor of this belief. Pulpitis, which develops at some later time, after operative intervention in a cavity, is not proof that the pulp is already infected. Such pulpitis might be hematogenous in origin (6), or this might be construed as proof that the method of sterilization was inadequate or that the medicament used was valueless or that no sterilization was attempted.

SUMMARY AND CONCLUSIONS

1. The dentin of 93 teeth, which had previously been treated with various medicaments and cultured twice, was cultured again 1 year later in order to find out if the bacteriologic status of the dentin had been altered.

2. Evidence has been presented to show that bacteria may still remain in the dentin after proper cavity preparation.

3. The cultures of all the cases, including controls, were predominately positive. The conclusion, from this data, is that sealing the dentin is not enough to sterilize it. The possibility of contamination is admitted which, however, indicates to a greater extent the effectiveness of antiseptic treatment of the dentin. Although not proven, the results point to the possibility that recurrence of decay under fillings is due to inadequate sterilization of the dentin before insertion of the filling. There is no other way, except leakage, to explain the presence in the dentin of organisms commonly associated with dental caries.

4. Morson's Kreosote, which had previously been pointed out as the best antiseptic in the series, continues to appear the best on the basis of this check-up. The search for the ideal antiseptic still continues.

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DENTAL CARIES AMONG SCHOOL CHILDREN OF BERGEN COUNTY, NEW JERSEY[1]

BION R. EAST, D.D.S. AND KURT POHLEN, PH.D.*

DeLamar Institute of Public Health, College of Physicians and Surgeons, Columbia University, New York, N. Y.

In public health practice and administration, factual knowledge concerning the prevalence of disease and the circumstances associated with its incidence is required to assure that the most effective measures will be undertaken in the public's interest at the lowest practicable cost. The same applies to disease with which the dental health administrator is concerned. The pathological condition which concerns the dental health administrator most is dental caries.

It is the usual practice in dental statistics to express the extent of dental caries, in a given sample of mouths, as the number of cavities, or as the number of teeth which have been affected by caries, per 100 persons. It has been assumed that the greatest possible knowledge of the incidence of caries and factors influencing the public's health by caries, is obtained from such data. We have pointed out that, in our opinion, such a formula is neither biologically nor logically correct.[2] We suggest that a health index of the teeth would give a better understanding of the distribution of caries among the *individuals* of a given sample, in that it would present a clearer picture of the number of children who were free from decayed, missing or filled permanent teeth as well as the number who were so affected at the various ages. We now present further evidence of the usefulness of data when expressed in the manner suggested in our former paper.

Recently data from a dental survey made in Bergen County, New Jersey were made available to us. This survey was made for a more immediately practical purpose than to supply us with data. However, these data offer an opportunity to illustrate the progress of tooth decay in children and to observe and study striking regularities in such progress and occurrence.

In *fig. 1* the average number of DMF[3] permanent teeth, per child, in the samples, according to sex and age is shown. The number of DMF teeth per child, increases, from year to year, with astonishing regularity. The increase is about one new DMF permanent tooth per child, per year. If we drew straight line curves through the mean values of the various ages, for the boys and girls, we would find that the curves are parallel, differ constantly, and the differences are of the same magnitude. This difference would be expected, because the

[1] Received for publication June 29, 1941.

* Now with the W. K. Kellogg Foundation, Battle Creek, Michigan.

[2] East, B. R. and Pohlen, K., Health Index of Teeth, *J. D. Res.* **20**: 559, 1941.

[3] Hereafter in this paper when referring to decayed, missing or filled teeth we will use the initials DMF.

teeth erupt earlier in girls than in boys and are therefore of a greater month-age at the same chronological age of the children. This similarity, the increase of the average number of DMF teeth per child, indicates that the forces which are associated with the prevalence of caries are, apparently, identical in each sex.

When the frequencies are stated as the number of children with 0, 1, 2, 3, 4 and so forth, DMF permanent teeth, per 100 children, the regularity of increase of carious teeth is emphasized. In Tables I and II, it is seen, that at six years of age, 41 and 52 per cent of the boys and girls, respectively, have no DMF permanent teeth. However 21 per cent of the girls and 15 per cent of the boys have as many as 4 DMF permanent teeth at the age of 6 years. At the age of 8 years only 4 per cent of the children, boys and girls, are free from DMF permanent teeth, while 11 per cent of the girls and 6 per cent of the boys have 5

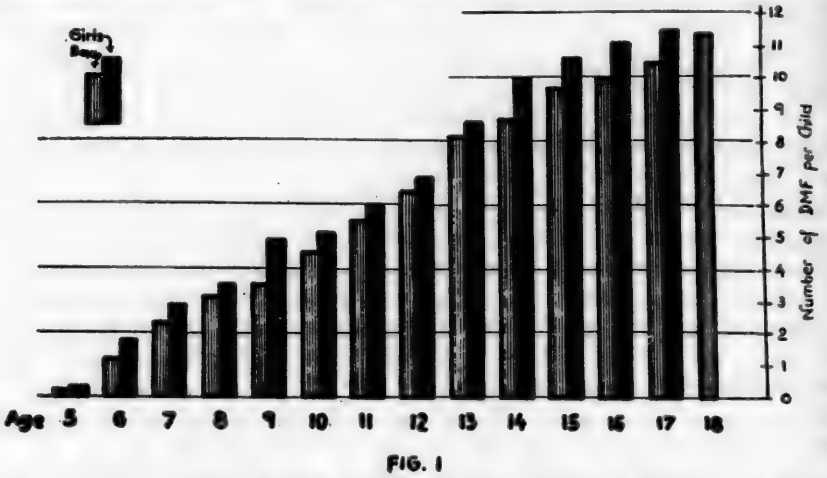


FIG. 1. Average number of DMF permanent teeth per child in different ages, Bergen Co., N. J.

or more DMF permanent teeth. When the small number of permanent teeth that are in the mouth at this age is considered, the percentages of affected teeth in the two sexes is impressive. At an age when only 8 permanent teeth are estimated to be present in the mouth, 50 per cent or more will have been attacked by caries. In Tables I and II striking examples of the frequencies of various numbers of DMF teeth in children are found. The number of children who have 4 DMF teeth increases rapidly until the children reach the age of 8 to 10 years. The number of children who have only 4 DMF teeth reaches its peak in the case of the girls at 8 and the boys at 10 years of age. For example, in the boys, aged 10 years, 3 per cent are free from DMF teeth, 2 per cent have 1, 6 per cent have 2, 8 per cent have 3 and 47 per cent have 4 affected teeth. The frequencies of children having 5, 6, 7, 8, etc., DMF teeth, respectively, decreases until only 1 per cent have as many as 12 DMF teeth at this age. It will be noted from the tables that there is regularly a marked reduction in the frequencies of

occurrence of 4 DMF teeth and those of 5 DMF teeth which does not occur elsewhere in the table. This is not surprising, because every dentist is aware of the greater relative amount of time and effort, in private or clinic practice, which must be devoted to restoration of the first permanent molars than to other permanent teeth. However, we believe that this particular fact has a more

TABLE I

Number of decayed, missing and filled permanent teeth (DMF) per 100 children of Bergen County, N. J.

Girls

	AGE IN YEARS AT LAST BIRTHDAY												
	5	6	7	8	9	10	11	12	13	14	15	16	17
Children with no DMF permanent teeth	85	41	16	4	3	2	5	6	1	—	—	—	—
1 DMF	7	10	6	5	5	1	1	1	—	—	—	—	3
2 DMF	4	10	21	13	9	6	5	2	2	2	2	4	—
3 DMF	—	17	15	13	15	8	4	4	4	—	2	—	—
4 DMF	4	21	31	54	44	34	22	20	10	6	3	1	5
5 DMF	.	1	9	7	9	17	15	6	5	2	5	—	—
6 DMF	.	.	1	1	9	10	7	15	5	7	2	—	5
7 DMF	.	.	—	—	4	8	6	7	16	8	5	6	8
8 DMF	.	.	—	3	1	7	12	7	10	9	13	14	8
9 DMF	.	.	1	—	—	2	10	9	16	19	15	13	—
10 DMF	.	.	.	.	—	2	4	6	6	12	13	11	18
11 DMF	.	.	.	.	—	—	5	3	4	12	7	3	5
12 DMF	.	.	.	.	—	1	1	6	5	1	7	5	5
13 DMF	.	.	.	.	1	1	3	—	2	8	2	14	8
14 DMF	.	.	.	.	.	1	—	5	5	3	5	8	13
15 DMF	.	.	.	.	.	.	.	1	2	3	7	10	10
16 DMF	.	.	.	.	.	.	.	1	2	1	5	1	—
17 DMF	.	.	.	.	.	.	.	—	4	1	—	4	—
18 DMF	.	.	.	.	.	.	.	1	—	—	—	4	3
19 DMF	.	.	.	.	.	.	.		—	5	2	1	5
20 DMF	.	.	.	.	.	.	.	.	1	—	5	—	3
21 and more DMF permanent teeth	.	.	.	.	.	.	.	.		2	2	1	3
Number of children examined	55	71	96	82	78	89	82	85	98	91	60	79	39

significant bearing on the probable genesis of dental decay than has been heretofore appreciated.

When the number of DMF permanent teeth, for each sex and age, are expressed as accumulated frequencies, we see further striking evidence of the regularity of the attack upon teeth by caries. In *figs. 2* and *3* the frequencies are plotted for each year of life, from 5 to 17 years inclusive, as the percentage of children, who have 0, 1–4, 5–8, 9–12, 13–16, 17–20, 21 and more DMF permanent teeth. These graphs demonstrate the group frequencies for the various ages. They also serve to illustrate cumulative frequencies, if the divisions are

read up from the lower limit for each age. We note a constant and steady decrease in the children having none or less than 4, 8, 12, 16 or 20 DMF teeth. The decrease is similar for both sexes.

In *figs. 4* and *5* are plotted the values for the different ages of the percentage of children with 0, 1, 2, 3, 4, 5 and more DMF permanent teeth. The values for the different ages have been connected by straight lines. The point of

TABLE II

Number of decayed, missing, and filled permanent teeth (DMF) per 100 children of Bergen County, N. J.

Boys

	AGE IN YEARS AT LAST BIRTHDAY												
	5	6	7	8	9	10	11	12	13	14	15	16	17
Children with no DMF permanent teeth	90	52	24	4	3	3	2	1	—	—	—	3	4
1 DMF	4	10	8	7	6	2	4	5	1	3	1	—	4
2 DMF	3	13	20	19	14	6	6	4	4	2	4	—	—
3 DMF	1	9	14	23	22	8	2	5	3	3	—	1	4
4 DMF	1	15	31	41	39	47	20	17	1	3	2	6	2
5 DMF	.	.	1	1	8	11	9	8	6	9	7	1	6
6 DMF	.	.	1	4	6	11	23	19	8	17	5	6	12
7 DMF	.	.	.	1	1	5	14	8	20	10	10	8	8
8 DMF	.	.	.	.	1	3	6	11	8	6	13	10	14
9 DMF	.	.	.	.	.	2	8	8	12	8	7	11	8
10 DMF	.	.	.	.	.	1	3	1	10	7	13	9	6
11 DMF	.	.	.	.	.	1	—	6	7	10	10	10	4
12 DMF	.	.	.	.	.	1	—	2	5	8	5	11	6
13 DMF	.	.	.	.	.	.	1	2	8	5	6	11	10
14 DMF	.	.	.	.	.	.	—	1	1	1	3	3	4
15 DMF	.	.	.	.	.	.	—	—	1	2	5	3	4
16 DMF	.	.	.	.	.	.	—	1	1	2	5	4	2
17 DMF	.	.	.	.	.	.	1	1	1	2	1	3	—
18 DMF	.	.	.	.	.	.	.	.	—	—	2	1	2
19 DMF	.	.	.	.	.	.		—	—	1	—	—	—
20 DMF	.	.	.	.	.	.	.	.	—	—	—	—	—
21 and more DMF permanent teeth	.	.	.	.	.	.	.	.	1	2	1	—	2
Number of children examined	99	86	74	80	89	93	65	90	75	110	85	73	51

intersection of the age line by the line connecting the DMF teeth frequency values, indicates the percentage of children of any age, having 0, 2, 3, 4, 5 and more affected permanent teeth.

The percentage of children who are *free* from DMF teeth, that is, the Health Index of the Biodenture,[4] decreases from year to year. This very rapid involve-

[4] We have suggested elsewhere that, for statistical purposes, "BIODENTURE" be used when referring to the natural teeth in a specified mouth. This term will describe not only the total erupted natural teeth in the mouth, but the organic system of teeth with all its major problems and metabolic, physiologic, and pathologic facts.

ment of the teeth of all the children by caries is the first impression one gets from the *figs. 4* and *5* as well as from *figs. 2* and *3*. The rapidly increasing percentage

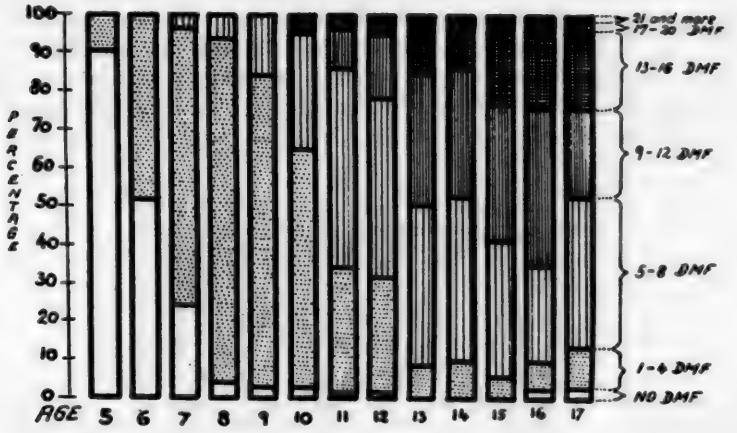


FIG. 2

FIG. 2. Boys with no and various number of DMF teeth among 100 examined in each age in Bergen Co., N. J. Percentage of boys having no, 1–4, 5–8, 9–12, 13–16, 17–20, and 21 and more DMF permanent teeth among each 100 boys examined, of the age from 5 to 17 years.

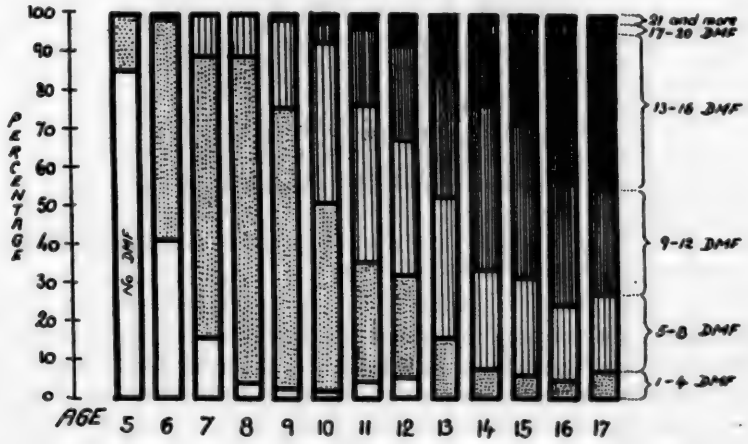


FIG. 3

FIG. 3. Girls with no and various number of DMF teeth among 100 examined in each age in Bergen Co., N. J. Percentage of girls having no, 1–4, 5–8, 9–12, 13–16, 17–20, and 21 and more DMF permanent teeth among each 100 girls examined of the age from 5 to 17 years.

of children who have multiple tooth involvement by caries is also noted in both groups of figures. However, another fact is noted. The graphs demonstrate

a biological fact, namely, that the various connecting lines can be easily smoothed into curves which fit the original points very well. We present these smoothed curves in *figs. 6* and *7*. We have drawn the smoothed curves by inspection, because we believe that samples of about 1000 boys and the same number of girls are not large enough to justify computation by mathematical formulae. But even these curves, drawn free hand, give some indication of the consistent character of the biological laws which govern the decay of permanent teeth. First of all we see two types of curves. The curves for 1, 2, 3, and 4 DMF teeth

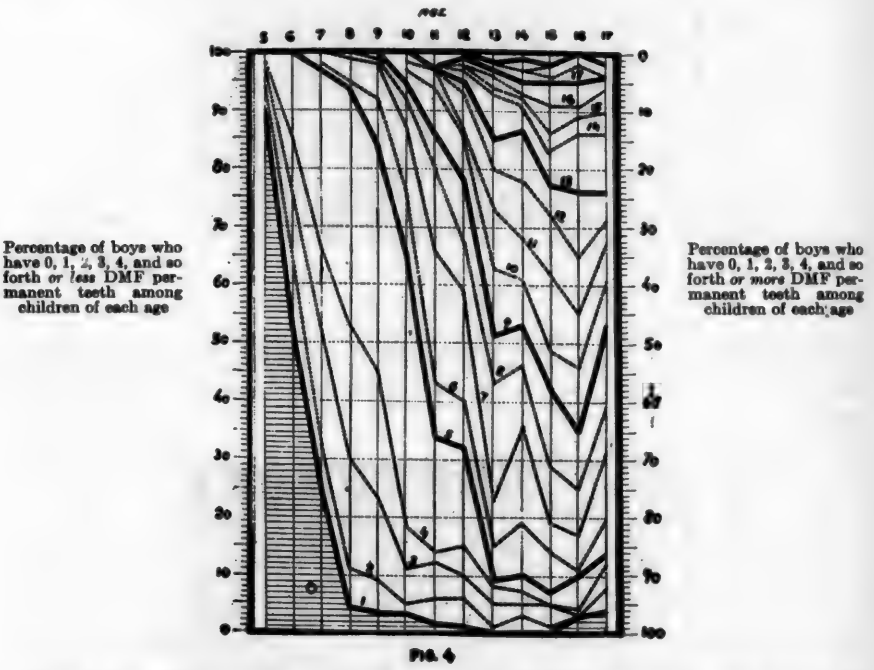

Percentage of boys who have 0, 1, 2, 3, 4, and so forth *or less* DMF permanent teeth among children of each age
Percentage of boys who have 0, 1, 2, 3, 4, and so forth *or more* DMF permanent teeth among children of each age
FIG. 4

FIG. 4. Boys without and with various numbers of DMF permanent teeth among 100 boys examined, aged 5 to 17, in Bergen Co., N. Y.

are hyperbolic, differing only in their exponents which express the amount of decrease from one year to the next, while the curves associated with 5, 6, 7 and more DMF teeth are "S" shaped. The differences in the shape of the curves suggest a difference in biological characteristics which may be related to resistance to dental caries. These curves differ in their individual exponents and also, perhaps, in the lower asymptote.

Our graphs emphasize what has long been observed, that the first permanent molars are the teeth most often affected by dental caries. However, it is also seen that the other teeth add considerably to the total per capita prevalence of dental caries in the older children. The particular point of interest is that teeth

other than the first molars respond to exposure to caries in a manner different from that of the first permanent molars.[5]

That the values obtained from the data are not due to chance is demonstrated by the regularity in the changes of slope of the various curves, and that both sexes give the same picture.

The diagonal distance between one curve and another approaches regularity and suggests that the slope and shape of the curves result from a biological law

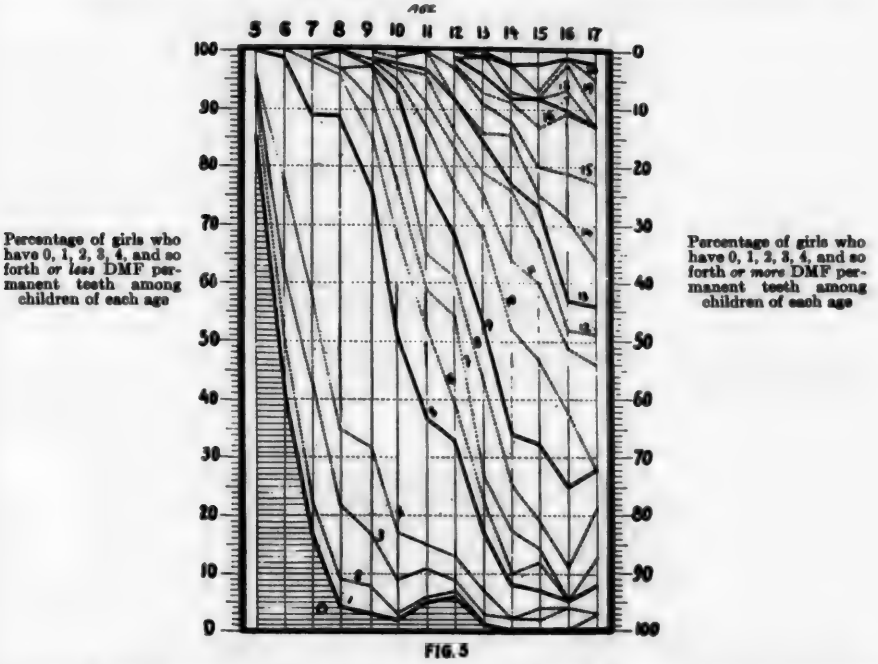

AGE
5 6 7 8 9 10 11 12 13 14 15 16 17
100 90 80 70 60 50 40 30 20 10 0
0 10 20 30 40 50 60 70 80 90 100
Percentage of girls who have 0, 1, 2, 3, 4, and so forth *or less* DMF permanent teeth among children of each age
Percentage of girls who have 0, 1, 2, 3, 4, and so forth *or more* DMF permanent teeth among children of each age
FIG. 5

FIG. 5. Girls without and with various numbers of DMF permanent teeth among 100 girls examined, aged 5 to 17, in Bergen Co., N. J.

which can be expressed mathematically. The dissimilarity between the diagonal distance between the curve representing 4 and 5 DMF teeth, and that between the other curves, again emphasizes the different biological response of the first permanent molars to attack by caries from that shown by other permanent teeth.

From these figures, one may estimate a life table of teeth comparable to the life table of a group of men. However, the two life tables will and should differ in some important respects. The life table of man is concerned with only one

[5] We appreciate that two or more permanent incisors erupt at the same time as the first permanent molars but believe we are justified in assuming that during early childhood these would seldom if ever materially influence the caries incidence of significant numbers of children.

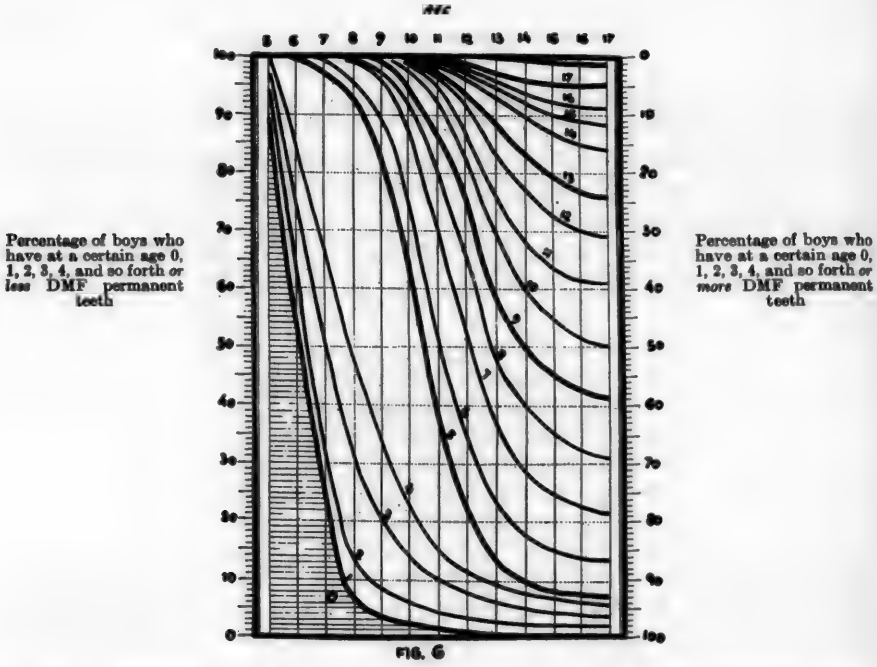

AGE
5 6 7 8 9 10 11 12 13 14 15 16 17
Percentage of boys who have at a certain age 0, 1, 2, 3, 4, and so forth *or less* DMF permanent teeth
Percentage of boys who have at a certain age 0, 1, 2, 3, 4, and so forth *or more* DMF permanent teeth
FIG. 6

FIG. 6. "Survivor table" of boys having a certain number of DMF permanent teeth, Bergen Co., N. J.

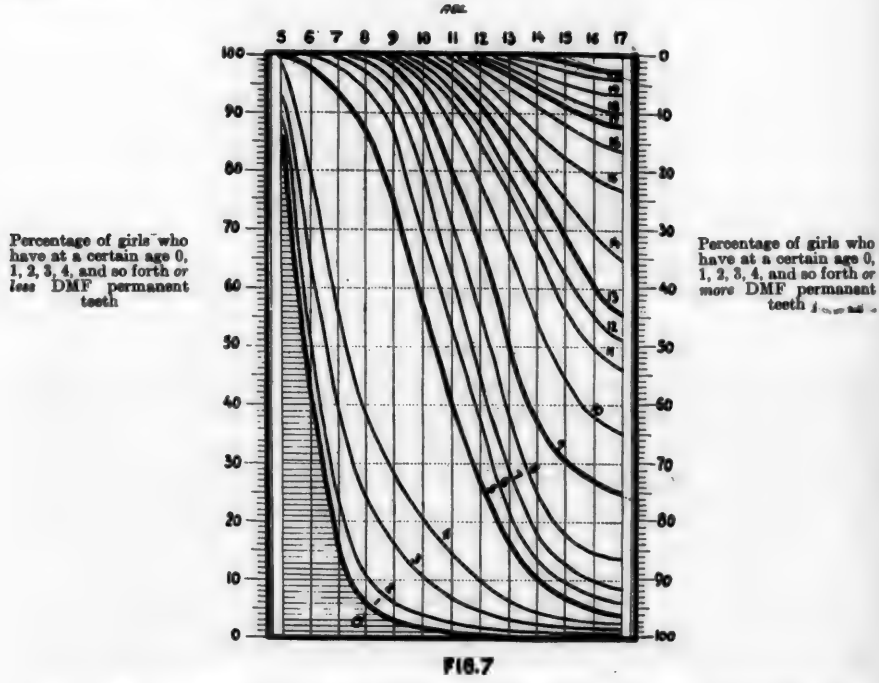

AGE
5 6 7 8 9 10 11 12 13 14 15 16 17
Percentage of girls who have at a certain age 0, 1, 2, 3, 4, and so forth *or less* DMF permanent teeth
Percentage of girls who have at a certain age 0, 1, 2, 3, 4, and so forth *or more* DMF permanent teeth
FIG.7

FIG. 7. "Survivor table" of girls having a certain number of DMF permanent teeth, Bergen Co., N. J.

event after birth, death. The individual is either alive or he is dead. In the life table of the teeth, in this particular problem, we are concerned with as many births (eruptions) and death (caries) as there are teeth. First, we have all sound teeth. The curve which represents this condition is similar to that which represents the data in the life tables of man. But after one tooth is DMF (in the life tables of man this would be the event of death), then there are many possibilities. This is shown in our *figs. 6* and *7*, where 2 per cent of the children of each sex have 20 or more DMF permanent teeth.

From our tables we can compute the probability of a child, of a given age, who has no DMF teeth, getting the first decayed tooth during the next year of life. We can compute all the probabilities that a child who has x DMF teeth, at the beginning of a certain year of life, will have x plus 1, x plus 2, x plus 3, etc. DMF teeth at the beginning of the next year of life.

We wish to point out that the data we present would apply only to the community of Bergen County, N. J. If it is desired to express the expectancy of dental caries in some particular community, based on such a tooth life table, it would be necessary to secure data from a representative sample of teeth of the peoples of various ages in that community. This follows because it has been shown that environmental factors, among other conditions, influence the incidence of caries. It would also follow that such data, and the curves based upon them, should reflect the environmental conditions of a community as measured by the effect on the prevalence of caries in the permanent teeth of children.

CONCLUSION

When the prevalence of dental caries follows such regular rules or empirical laws as are illustrated in our data and graphs, we feel justified in speculating with some confidence upon the causes of dental caries. The difference in susceptibility of the first permanent molars and other permanent teeth, to dental caries and the regularity of the two types of curves of caries prevalence associated with the different teeth, leads us to suggest that the simple explanation of caries as resulting entirely from external causes within the mouth or in the general environment is incompatible with the facts. We admit that merely external agents or other external causes may and often do affect with some regularity the occurrence of caries, but we believe that such regularities would be expressed by curves of different shapes and would show very different tendencies of increase during life, than is demonstrated by the hyperbolas of 1, 2, 3, and 4 DMF teeth.

We believe, if we were dealing in dental caries with external causes exclusively that all the curves would be of the same general shape and that they should approach the "S" shape and not be hyperbolic as they are in the case of the first permanent molars.

DENTAL CARIES RATES AND MOUTH-AGE OF TEETH[1]

BION R. EAST, D.D.S.

DeLamar Institute of Public Health, College of Physicians and Surgeons, Columbia University, New York, N. Y.

Generally speaking, the principal conditions determining the extent of chemical reactions are the amounts of the reacting substances brought into contact and the length of time they are allowed to react. Considering this well known law of mass action, it would seem to follow, if, as some believe, dental caries is the sole result of chemical reactions between tooth substances and bacteria-produced acids, that the greater the number of teeth and the longer they have been exposed to such attacks, the greater should be the caries attack rates.

Palmer, Klein and Kramer, among others, recognized the necessity of considering the total number of erupted teeth and their accumulated post eruptive mouth age in rationalizing caries experience among a given population. They determined these values for children residing in Hagerstown, Maryland (1). Like determinations have been made for children residing in Chicago, Illinois (2).

For the purpose of this discussion it has been assumed that the values for the accumulated post eruptive mouth age of the permanent teeth of the children of Hagerstown would apply to children of the same sex and age residing in other areas of the United States. However, this assumption should be tested by data from many localities before it is accepted for more than a working hypothesis.

The term *exposure unit* is suggested to designate a year of post eruptive mouth age of an individual tooth. In Table I the accumulated number of exposure units for the teeth of the children of Hagerstown and Chicago are given. It will be noted that the values for comparable age-sex groups do not differ greatly for the children of the two cities.

The mean number of decayed, missing or filled (D.M.F.) permanent teeth per child among groups of children of Topeka, Kansas; Chicago, Illinois; and cities of Bergen County, New Jersey, was determined from data collected in surveys of school children of those localities.[2] The mean values are given in Table II. Like values for Hagerstown children are also given.[3] The relation of the mean number of caries attacked permanent teeth and the total mean number of accumulated years the teeth had been in the mouth among the various age-sex-

[1] Received for publication August 4, 1941.

[2] The data were made available through the courtesy of Dr. Leon Kramer, Kansas State Department of Health; Dr. Carl Greenwald, Chicago Department of Health; and Dr. J. M. Wisan, New Jersey State Department of Health, respectively.

[3] Adopted from original publication, Public Health Reports, Vol. 53, No. 19, May 13, 1938.

TABLE I

*Accumulated exposure units (accumulated mouth age of erupted teeth) of permanent teeth of 1929 Chicago, Illinois, and 4416 Hagerstown, Maryland, school children**

CHRONOLOGICAL AGE (YEARS)	BOYS		GIRLS	
	Mean Number Accumulated Exposure Units of Permanent Teeth Per Child			
	Locality			
	Chicago	Hagerstown	Chicago	Hagerstown
6.5	2.9	1.9	3.3	3.2
7.5	9.2	7.4	10.5	9.7
8.5	18.9	16.9	19.5	20.1
9.5	27.2	29.2	33.5	33.5
10.5	46.7	44.1	50.5	50.1
11.5	63.9	62.5	70.9	70.7
12.5	88.1	84.9	93.6	94.6
13.5	111.3	109.9	116.5	120.8
14.5	134.0	137.9	139.4	148.3

* The Hagerstown data are from Palmer, Klein and Kramer; Studies on Dental Caries III, A Method of Determining Post-Eruptive Tooth Age, *Growth*, 2:149–158, 1938.

TABLE II

Mean number of decayed, missing or filled (D.M.F.) permanent teeth among 820 Topeka, Kansas; 4416 Hagerstown, Maryland; 1929 Chicago, Illinois; and 1658 Bergen County, New Jersey children

CHRONOLOGICAL AGE (LAST BIRTHDAY)	BOYS				GIRLS			
	Locality							
	Topeka	Hagerstown	Chicago	Bergen	Topeka	Hagerstown	Chicago	Bergen
6	.34	.25	.50	1.2	.17	.33	.63	1.7
7	.38	.58	1.8	2.3	.43	.86	2.0	2.8
8	1.3	1.1	2.6	3.1	.89	1.3	2.9	3.5
9	1.8	1.8	3.2	3.5	1.5	2.3	3.6	4.9
10	2.2	2.4	3.2	4.5	1.9	2.6	3.7	5.1
11	2.4	2.8	3.4	5.5	2.2	2.9	4.1	6.0
12	3.1	3.6	4.2	6.4	3.3	3.7	5.2	6.8
13	4.2	4.0	4.8	8.2	3.1	5.1	5.4	8.6
14	—	5.1	5.8	8.7	3.8	5.4	5.7	10.0

locality groups was determined by dividing the mean number of D.M.F. teeth by the appropriate number of accumulated exposure units. The resulting rates are given in Table III.[4]

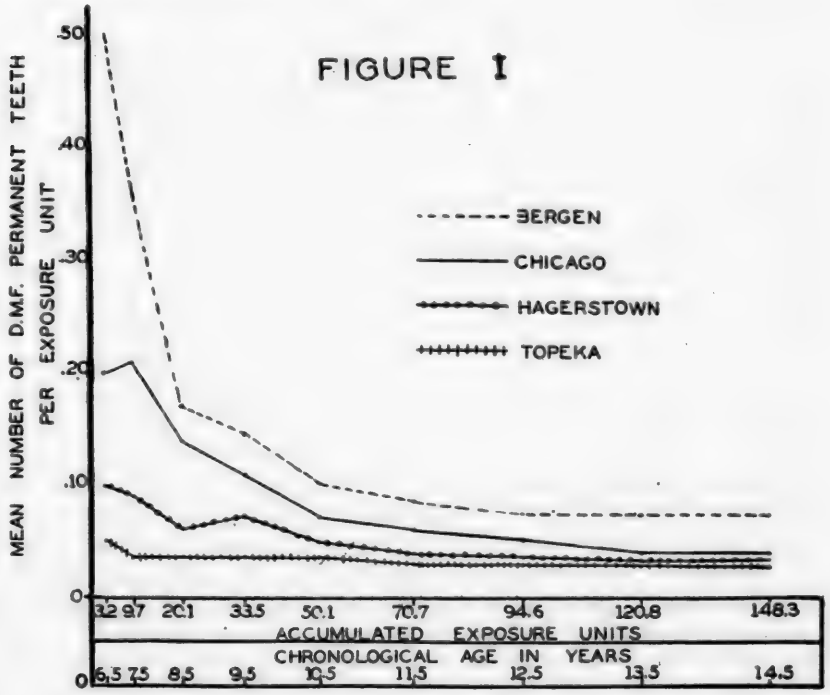
[4] It is recognized that variation in criteria and methods of recording may account for some of the differences observed in the original DMF values. However, such variation would not influence the shape of the individual curves because the criteria are assumed to be constant in each locality. It is also doubted that differences in criteria, if any, could account for all the relative differences in the rates among the different groups.

TABLE III

Mean number (caries prevalence rate) of D.M.F. permanent teeth per exposure unit among white children of nine chronological age groups residing at four localities of the United States*

MEAN CHRONOLOGICAL AGE (YEARS)	BOYS				GIRLS			
	Locality							
	Kansas	Hagerstown	Chicago	Bergen	Kansas	Hagerstown	Chicago	Bergen
6.5	.18	.13	.26	.63	.05	.10	.20	.53
7.5	.05	.07	.24	.42	.04	.09	.21	.36
8.5	.07	.07	.15	.18	.04	.06	.14	.17
9.5	.06	.06	.11	.12	.04	.07	.11	.15
10.5	.05	.05	.07	.10	.04	.05	.07	.10
11.5	.04	.04	.05	.09	.03	.04	.06	.08
12.5	.04	.04	.05	.07	.03	.04	.05	.07
13.5	.04	.04	.04	.07	.03	.04	.04	.07
14.5	—	.04	.04	.06	.03	.04	.04	.07

* Decayed, Missing or Filled.



In *fig. 1* the rates for the girls of the 4 localities are shown graphically. The several values for each group have been connected by straight lines. The resulting rough curves demonstrate the differences as well as the similarities in

caries susceptibility of the teeth of the girls living in different localities. For example, among the Bergen County girls of 6½ years of age, the caries prevalence rate among the permanent teeth was .53 teeth per year of accumulated post eruptive mouth age. Among the Kansas girls of the same age group, the rate was only .05. The curves apparently demonstrate that, among the children composing the groups of this study, the number of permanent teeth that have been attacked by caries is not in direct proportion to the total accumulated post eruptive mouth age of the teeth. A negative relationship seems to exist.

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1. Palmer, C. D., Klein, H., and Kramer, M., Studies on dental caries III. A method of determining post-eruptive tooth age. *Growth*, **2**: 149–158, 1938.
2. Greenwald, C., and East, B. R., Eruption tables of permanent teeth of Chicago school children. *Illinois D. J.* **10**: 361, 1941.

EFFECT OF DIETARY FLUORINE IN DELAYING DENTAL CARIES[1]

J. F. McCLENDON AND W. C. FOSTER

Research Laboratory of Physiology, Hahnemann Medical College, Philadelphia, Pa.

It has been shown that fluorapatite, $FCa_4P_3O_{12}$, is absolutely insoluble in saliva of pH 5.5 to 7 (Robb, Medes, McClendon, Graham and Murphy, 1921) and that 0.5 per cent and 0.75 per cent powdered fluorapatite (McClendon, 1923) added to the following diets improved the bones and teeth: *diet 255*—66 parts white flour, 5 casein, 5 lactalbumin, 2 $CaSO_4$, 2 NaCl, 20 butter fat; *diet 256*—diet 255 plus 1 per cent yeast; *diet 281*—diet 255 with cottonseed oil instead of butter fat; and *diet 282*—diet 281 plus 1 per cent yeast. The x-ray pictures of these rats have been retained.

This problem was given to W. D. Armstrong (1932). Armstrong designed a glass still for SiF_4 distillation (1933, a) and later had this still reproduced in platinum. Since Willard and Winter (1933) showed that hydrofluosilic acid may be quantitatively distilled and thus avoid the rigid anhydrous technique required for SiF_4, Armstrong (1933, b, 1936) improved their method.

Hoppert, Webber and Canniff (1932) on observing that the diet of 60 parts coarse yellow corn meal, 30 whole milk powder, 6 linseed meal, 3 alfalfa meal and 1 NaCl produced caries in rats, attributed the caries to the coarse particles of corn. We experienced some failures in trying to produce caries in mice on this diet and attributed them to the geographic variation in fluorine content of foodstuffs. But since no one had accurately determined the fluorine content of such a diet we developed a method for dietary fluorine (McClendon and Foster 1941, a) and began the study of the effect of fertilizers on the fluorine content of corn. In collaboration with G. C. Supplee we began a geographic study of the fluorine content of milk.

In the meantime a number of papers (Am. Dent. Assn., 1941) on the relation of fluorine to dental caries have appeared. Armstrong and Brekhus (1938) were the first to show with accurate analytical methods that human enamel from sound teeth contained more fluorine than enamel from carious teeth. Armstrong (quoted by Hodge, Luce-Clausen and Brown 1939) was the first to determine the fluorine content of rats' teeth. The investigators of the relation of fluorine to dental caries may be classed according to their conclusions. The following concluded that the fluorine acts as a constituent of fluorapatite in the teeth: Gruner, McConnell and Armstrong (1937), Armstrong and Brekhus (1938), Cox, Matuschak, Dixon, Dodds and Walker (1939) and McClendon and Foster (1941, b). Furthermore Dean, Fay, Arnold and Elvove (1941) have shown that 50 children of Bauxite, Ark., who had had high fluorine drinking

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water only the first 2 to 10 years of life but had been drinking fluorine-free water for the past 12 years had only half the caries rate of the 45 children of Benton, Ark., who had drunk fluorine-free water all of their lives. This continued effect of fluorine for 12 years after taking was probably due to its storage in the teeth. On the other hand Miller (1938) and McClure (1941) conclude that the action of fluorine is anti-enzymatic on the bacteria in the mouth. Finn and Hodge (1941) state that "the mechanism of the inhibiting or protective action of fluorine is unknown." For this reason and also because many of these workers have given fluorine to rats in concentrations that would be toxic to humans, and since we have been the only workers to analyze the fluorine content of the rats' diet, the following experiments are recorded.

EXPERIMENTAL

In searching for a caries-producing diet we dehydrated fresh teeth in methanol and cleared them in benzyl benzoate, pumping out any remaining methanol or any air bubbles with the air pump. These were studied under the binocular microscope. Some were made into permanent mounts by mixing the benzyl benzoate with solid Canada balsam or "nevillite" (clarite-x) or polymerizing "plexiglass" in the benzyl benzoate while the teeth were mounted on a glass slide under a cover glass. But due to the pressure of chemical work, in later experiments, the teeth were examined with the binocular microscope immediately after the death of the rat and the 1st, 2nd or 3rd, right or left, upper or lower carious molar teeth recorded. In case the teeth were to be analyzed for fluorine a fur ther record was filed by photographing the teeth through one of the eyepieces of the binocular microscope while the focus was verified through the other eyepiece. The other teeth were stored.

The control caries-producing diet was 400 grams brown rice,[2] 400 grams yellow corn,[3] 140 grams skim milk powder,[4] 90 grams "Mazola" corn oil and 10 grams Baker's Analyzed NaCl. The corn and rice were cracked in a mill and the dry ingredients well mixed before adding the oil. Analysis of the first sample gave 0.45 ppm.[5] fluorine but later analyses of this diet gave 0.3 ppm. fluorine. Twenty-one day old Wistar strain rats developed caries on this diet in 40 days. These rats are designated "controls." Litter mate controls were compared with the rats given "caries-delaying" additions to the diet. Since, however, no litter differences were noted, 70 controls were tabulated in the second column of Table I and duplicated in the second column of Table II. All rats were started at weaning age (21 days) and their teeth examined only when they were killed or had died.

When 2 per cent coarsely ground bone meal was added to the diet there were no caries in 60 days but an average of 3 carious teeth per rat in 230 days. This experiment was designed to test whether the caries were due to coarse grinding

[2] Gift of Southern Rice Sales Co. Inc., N. Y.
[3] Gift of the N. J. Agricultural Experimental Station.
[4] Gift of G. C. Supplee, The Borden Co., Bainbridge, N. Y.
[5] ppm. = parts per million.

but is inconclusive due to the small percentage of bone meal and fluorine (0.316 ppm. fluorine).

It may be seen from the data in Table I that small additions of ground bone or tooth or salts of calcium or phosphoric acid lessen the number of carious teeth when compared with controls of the same age. The same is true of cod liver

TABLE I

Carious teeth per rat

DAYS ON DIET	CONTROL (70 RATS)	2 PER CENT BONE	1 PER CENT HORSE TEETH	1 PER CENT $Ca_3(PO_4)_2$	1 PER CENT Na_2HPO_4	1 PER CENT $CaCO_3$	0.2 PER CENT C.L.O.
40	1.5						
75	3				1.5		
90	3.3		2				
100	3.5				2	3.4	
150	4						3
160	4						
230	5	3		4.3			

TABLE II

Carious teeth per rat

DAYS ON DIET	DISTILLED	DRINKING WATER				
		+1.3 ppm. F	+4.5 ppm. F	+6.8 ppm. F	+9 ppm. F	+10 ppm. F
40	1.5					
75	3					0
90	3.3		0	0		
100	3.5				0	
150	4	2	1			
160	4		2	0.6		2
230	5		3	2	2	

TABLE III

Percentage of fluorine in teeth and bone

WATER	ENAMEL	DENTIN	FEMUR
Distilled	0.0060	0.0069	0.0035
+NaF	0.0250	0.0412	0.0400
An increase of about	4 times	6 times	11 times

oil. These diets by analysis did not contain more than 0.33 ppm. fluorine, so the slight improvement was probably not due to fluorine. In contrast to this slight improvement compare the effects of fluorine (as sodium fluoride) in the drinking water in Table II.

Drinking water containing 9 or 10 ppm. fluorine produced striations of the enamel of the incisor teeth. It seems evident from the data in Table II that

even such small amounts of fluorine that have been reported in some municipal drinking waters delay the appearance of gross dental caries in rats. The average body weight of rats on fluorine in drinking water at 230 days was 259 grams and controls 176 grams.

Instead of killing all of the rats at the same time, some were kept alive as long as possible. Of 62 rats on the control diet and distilled water, 18 (or 29 per cent) died within 230 days whereas of 27 rats on the same diet but with NaF added to their drinking water, 3 (or 9 per cent) died within 230 days.

The addition of NaF to the water increased the percentage of fluorine in dentin and enamel as well as bone (Table III).

CONCLUSIONS

The cracked corn and rice diet produces caries in rats' teeth which increase in number with the days on the diet. Small improvements in the diet in the way of ground bone, teeth, calcium salts, phosphates or cod liver oil seem to delay slightly the onset of caries. Small additions of fluorides to the drinking water delay the onset of caries to a much greater extent. It seems possible that if rats lived long enough they would develop caries on any diet.

SUMMARY

Evidence collected for 20 years points to fluorine as essential to the teeth although the public interest was centered on dental fluorosis. Up to 10 ppm. fluorine in drinking water delayed dental caries produced by a cracked cereal diet containing 0.3 ppm. fluorine. Small dietary additions designed to improve the calcium phosphate metabolism slightly delayed dental caries. These additions were not all fluorine-free but did not add more than 0.03 ppm. of fluorine to the diet.

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PRODUCTION OF GRADED MOTTLING IN MOLAR TEETH OF RATS BY FEEDING OF POTASSIUM FLUORIDE[1]

VIRGIL D. CHEYNE, D.D.S., PH.D.

Indiana University School of Dentistry, Indianapolis, Indiana, and School of Medicine and Dentistry, The University of Rochester, Rochester, New York

The molars of the rat offer excellent material for the testing of experimental tooth destruction. The animal breeds freely, is cosmopolitan, and can be economically retained in large numbers for laboratory purposes. The development of the teeth and the characteristics of the formed structures are similar to those of the human in many respects (Mellanby, 1939; Hoffman and Schour, 1940). The occlusion, cuspal form, roots and attachments all bear a marked resemblance. The enamel and dentin are limited in growth and are subject to dental caries with all of the attending pathosis found under similar conditions in human teeth (Rosebury, 1938). As the caries spreads, there is dentinal penetration and ultimate pulpal degeneration.

We have used the rat extensively for investigating the mechanism of dental caries inhibition by fluorine (Cheyne, 1940 a, 1940 b). In such animals a "standardized" type and degree of dental caries was produced. In the course of these studies we found it necessary to effect mottling in the molars of some of our experimental groups comparable to mottling observed in the teeth of humans observed in various fluorine endemic areas in the United States (McKay and Black, 1916; Dean, 1934). Since animals receiving fluorine in their diet during formation of the teeth deposit the element in the dentin and enamel in high concentrations, (Hodge, Luce-Clausen and Brown, 1939) this offered an opportunity for studying, simultaneously, the interrelations between dental caries and fluorosed dental tissue. The present report is concerned with that phase of the investigations which describes the amount of fluorine required to produce hypoplastic defects, the period in the life span of the animal wherein the fluorine was fed, and the observed effects. Graded amounts of the elements were given and correlated with the severity of mottling which resulted.

In 1931 Smith, Lantz and Smith, and Churchill, working independently, first demonstrated that the mottling of teeth was caused by the toxic incorporation of fluorides in the drinking water during the calcification process. Since then Smith and Lantz (1933) and others (Bethke, Kick, Hill and Chase, 1933) have experimentally produced a similar condition in the teeth of animals under the influence of a high fluorine diet. For the most part, however, the teeth affected

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have been those of persistent growth. Unfortunately, such teeth and the teeth of animals other than the rat are of no value for use in studies concerned with experimental dental caries.

The minimum quantity of fluorine required to produce mottled enamel in rat teeth has not been accurately determined. McClure (1939) calculated the amount necessary for humans. In this regard he stated that the average child of 15 kilogram living in a fluorine endemic area where the water contains 2 to 3 p.p.m.[2] fluorine would consume between 0.15 to 0.30 mgm. of fluorine per kilogram of body weight daily. This is sufficient to cause mottling.

Only two reports have appeared in the literature describing the macroscopic effects of quantitative doses of fluorine on the molar teeth of rats. Neither, however, describes the production of defects proportional in severity to the amount of fluorine administered. In the first, Cox, Matuschak, Dixon and Walker (1939) mention a diffuse milky mottling in the enamel of the first and second molars in 2 animals, each receiving a total of 128 micrograms of fluorine during the suckling period (21 days). In a third animal, fed 256 micrograms for the same period of time, the first and second molars showed what was described as corroded enamel. Later, from the same laboratory, Dixon and Cox (1939) reported that they observed a faint milkiness in the third molars of a colony of rats fed 250 p.p.m. sodium fluoride in the ration from weaning until 8 weeks of age. Photographs of these lesions were not published.

The gross appearance of human mottled enamel was accurately described by McKay and Black (1916) several years before the etiological agent was known. It is a hypoplastic condition represented by an irregular distribution of grayish white blotches of chalky areas over the tooth surface. The first, or mildest detectable, symptom is a whitish discoloration at the time of eruption. In teeth affected more severely the defects take the form of irregular pits; on others, an incomplete calcification of cusp tips. Brownish discoloration may or may not occur later. In extreme cases, the entire tooth surface is pitted and destroyed. The various types have been studied in endemic areas and accurately classified by Dean (1934).

Knouff, Edwards, Preston and Kitchen (1936) have pointed out that the placenta of the dog is impermeable to the fluorine ion at low concentrations, but may be permeable at higher concentrations. Mottled deciduous teeth in children have been reported (Smith and Smith, 1935), but most investigators agree that these teeth are affected only infrequently. Inasmuch as deciduous teeth are believed to calcify chiefly before birth, whereas the permanent set undergoes calcification following birth, the resistance of the placenta has been offered as an explanation of the relative immunity. For the present investigation, therefore, it has been necessary to determine the approximate postnatal age period covered by the development of the crown of rat molars if we were to produce mottling in that portion of the tooth. For this purpose we have used the data given in Table I. The figures were collected by Hoffman and Schour (1938; 1940) and have specific reference to the formation of the lower molars. In most cases, as

[2] P.p.m. = parts per million.

these investigators state, calcification and eruption of the molars of the lower jaw are slightly in advance of the upper.

Calcification of the enamel and dentin of the lower first molar begins the first day after birth. This tooth precedes the upper first molar as well as the lower second molar, and the first and second molars in both jaws calcify well in advance of the third molars. It can be noted that the teeth are slow to form. Elongation due to primary dentin deposition is not complete in the first molars until the animals are approximately 83 days old. The attachment apparatus for the third molars is not complete until about 115 days after birth.

Our eruption data is in agreement with that of Addison and Appleton (1921), who state that the first molar erupts at the nineteenth day, the second molar about the twenty-first day, and all teeth being in the mouth by the thirty-fifth day. These figures are added to Table I for the purpose of completeness.

TABLE I

*Postnatal chronological development of the fixed dentition of the rat**

DEVELOPMENTAL PROCESS	MOLAR 1	MOLAR 2	MOLAR 3
Beginning of apposition of primary dentin (enamel apposition occurs 12–24 hours later)	20th day in utero to birth	2nd–3rd day post-natal	13th–14th day post-natal
Beginning of calcification	Birth to 1st day	2nd–3rd day	15th day
Completion of appositional growth in crown	10th–11th day	12th–13th day	19th–21st day
Beginning of bifurcation of roots	11th–13th day	14th–16th day	24th day
First appearance in oral cavity	19th day	21st–22nd day	35th day
Cessation of root elongation by means of primary dentin	80th–83rd day	90th–95th day	110th–115th day

* The data in this table are abstracted from the investigations of Hoffman and Schour (1940). Hoffman and Schour in turn compounded the findings of Addison and Appleton (1921), Glasstone (1938), Mellanby (1939) and own investigations (1938, 1940).

EXPERIMENTAL METHODS

Animals. The present study is based upon observations made on 43 rats (*Mus norvegicus albinus*). Three additional animals were used as controls. Members of the colony were the offspring of 8 litter-mate mothers and were chosen because of their healthy appearance and approximate equality in birth weight. Opposite sexes were paired for fluorine dosage trials.

Method of fluorine administration. Early attempts to produce mottling of rat molars in our laboratory by the administration of fluorine to newborn animals resulted in a high mortality. We overcame this difficulty in the present trials by feeding the mother 600 p.p.m. fluorine as potassium fluoride throughout her pregnancy and lactation. Although we are unable to explain how this regime influenced the young, they were better able to withstand the postnatal administration of the drug. Fluorine thereafter was administered to the young on the day of birth and continued until the end of each test period. By premedication

of the mothers, concentrations as high as 550 p.p.m. have been given to the offspring. By altering the amount from 150 to 450 p.p.m. during the molar calcification period variations in the degree of mottling have been successfully accomplished. Continuation of the feeding until the animals were 30 days of age caused a uniform involvement without loss of contour of the surfaces of all molars in concentrations approaching 300 p.p.m.

The mothers of experimental animals were given oral doses of fluorine daily in aqueous dilutions with a medicine dropper. A 1 cc. syringe equipped with a blunted hypodermic needle was used for the young. The tip of the needle was placed on the posterior part of the tongue and the required amount ejected. The stimulation elicited a "sucking response" from the animal and it swallowed the dose.

A basal diet was given to the mothers and to the young after weaning. It was provided in adequate quantities and the source renewed at frequent intervals in order to insure freshness. Weights of the animals were taken at birth and again at the time of death.

Study of material. A study of the oral cavity of the animals was carried out according to the following plan: Most of the rats were chloroformed between 33 and 40 days of age. The teeth were prepared by freeing the jaws of all adhering soft tissues and placing them in 10 per cent formalin. After 24 hours the mandibles and maxillae were removed and split. The teeth were dried and examined by reflected light under a binocular microscope of approximately 30 × magnification. The affected areas on the teeth were noted and tabulated accurately according to location on an examination form. Later the forms were arranged in sequence according to the amount of fluorine given. Typical cases were selected and photographed. If the animals died before the molar teeth had erupted, the procedure consisted of removing the soft tissues and overlying bone around the teeth. The teeth were then examined individually for evidences of fluorosis. For the microscopic study, alternating ground and decalcified sections were prepared for each jaw. Midpulpal sections involving the 3 molars comprising a molar segment were prepared for study except in cases where the teeth were removed individually.

RESULTS

Table II gives a synopsis of the data on the concentration amount of fluorine administered, the duration of the feeding period, and the condition of the molars in 18 representative rats. Three arbitrary group divisions are made using as a basis the gross effect of doses upon the teeth. It is apparent from the table that the teeth showed graded defects, the severity of which was roughly proportional to the concentration of the drug administered. Moreover, a correlation of the description of the gross findings with the age period of fluorine feeding indicates that the location of the damage accords with that part of the tooth in the process of calcification at that time.

Macroscopic findings: The animals fed fluorine in concentrations of 150 to 200 p.p.m. for short periods of time evidenced little, if any, change in the char-

TABLE II

History of 18 rats used in determining the optimum amount of fluorine fed for the production of mottled molars

GROUP NUMBER AND DESCRIPTION	RAT NUMBER AND SEX	WEIGHT AT BIRTH IN GRAMS	WEIGHT AT DEATH IN GRAMS	AGE AT BEGINNING OF FEEDING IN DAYS	AGE AT DEATH IN DAYS	DAYS OF FLUORINE FEEDING	DAILY CONCENTRATION OF FLUORINE AS POTASSIUM FLUORIDE (P.P.M.)*	GROSS FINDINGS IN TEETH
I Doubtful to mild mottling	6101♂	4.7	36	1	34	14	150	No mottling
	6103♂	4.6	33	1	34	4	200	No mottling
	6104♂	4.8	37	1	34	30	150	All molars in animals 6104 and 6108 were mildly mottled. Molars in animal 6105 were doubtfully affected
	6105♀	4.4	36	1	34	30	150	
	6108♀	4.7	36	1	34	30	150	
	6109♀	4.5	41	1	40	30	250	Uniform mild mottling
II Definite mottling. No loss of surface contour	6112♂	4.9	30	1	34	3	300	Tips of mesial cusps of lower first molar showed mild mottling
	6114♂	5.1	43	1	40	10	300	Whole crown of lower first molar, occlusal ⅔ of lower second molar, and upper first molar were mildly mottled. The upper second molar showed mottling in the occlusal ½.
	6116♀	4.8	33	1	34	15	350	Crowns of all first and second molars were mottled and occlusal ½ of lower third molars were affected
	6118♀	4.8	36	1	34	15	300	
	6123♀	5.0	34	1	33	30	300	All molars in dentition were mottled; moderately severe in type
	6124♂	5.1	30	1	33	30	300	All molars in dentition were mildly, but definitely affected
	6125♂	5.0	31	1	33	30	300	
	6126♂	5.4	35	1	33	30	300	
III Corroded mottling. Severe in type (general acute toxicosis)	6132♀	5.2	7·3	1	4	4	450	Tips of cusps of lower first and second molars showed moderately severe hypoplasia. Crowns only partially formed
	6135♀	4.6	15	1	14	14	450	Death of animal; not examined
	6137♀	5.0	30	1	33	30	450	All molars in dentition were severely affected
	6141♂	5.2	47	1	46	30	500	All molars in dentition were severely mottled, chipped and abraded

* p.p.m. equals parts per million of fluorine to total food consumption by weight. The calculation is based upon the assumption that a 200 gram rat eats about 15 grams of food per day, and the corresponding dosage computed according to the weight of the smaller animal. The animals were weighed each day before fluorine was given.

acter of their teeth. When they were given 300 p.p.m. or more of fluorine, however, there was a uniform hypoplasia, usually of the mild to moderately severe type. High doses (450 to 550 p.p.m.), combined with continued administration, resulted in corrosion of the teeth and a generalized acute toxicosis of the animal. Increasing doses, above 400 p.p.m., caused a proportionate increase in mortality, but in those that survived, the dentition was uniformly and severely affected. Two illustrations of jaw segments from a control animal and segments from 3 animals receiving progressively larger doses of fluorine are presented to show these changes.

Addison and Appleton (1921) observed that the summits of the cusps of the rat molar normally are denuded of enamel. The remainder, however, is covered by a thick layer. These observations have been confirmed by studies of preerupted teeth. These enamel-free areas are shown in *fig. 1*, which is a photomicrograph of a ground section through the mandibular segment of a normal control animal sacrificed soon after eruption of all molars. A gross photograph of the opposite segment is shown in *fig. 2*. On inspection, the enamel covering the remainder of the crowns of the teeth of the control group was observed to be normal in every respect. The figure shows the surfaces to be smooth and nearly translucent. The color was creamy white by reflected light, showing only a slight tinge of yellow.

Fig. 3 illustrates a mandibular jaw segment taken from one of the animals that received large enough doses of fluorine to cause superficial linear markings on the surfaces of the teeth. On gross inspection, these areas were of a mild "glazed glass" appearance and were smooth to the explorer tine. The anatomical contour of the teeth was not destroyed. Per cent of dosage: 600 p.p.m. fluorine fed as a potassium fluoride daily to the mother during pregnancy and lactation; young, 300 p.p.m., 1 to 30 days of age.

Fig. 4 shows a more pronounced mottling of the pitted and stained type. Although of sufficient density to retain the outlines of normal tooth contour at the time of eruption, the occlusal four-fifths of the crowns were a dirty yellow; the chalky spots were easily penetrated by the explorer tine. The enamel was chipped in the region of the cusp summits where mastication stresses were brought to bear. Per cent of dosage: 600 p.p.m. fluorine fed as potassium fluoride daily to the mother during pregnancy and lactation; young, 350 p.p.m., 1 to 9 days of age.

With the higher levels of fluorine feeding, macroscopic hypoplasia with pitting was observed in the enamel of all teeth of animals that survived the thirty day feeding period. An example of this is shown in *fig. 5*. A large proportion of tooth surfaces is affected, softening of the enamel is extensive, and large patches of dentin are denuded. Friability of the enamel was evidenced by many chipped and broken teeth after the animals masticated solid food for a few days. Discoloration frequently appeared in the denuded areas of animals allowed to live for 1 or 2 weeks after weaning. In rats with marked hypoplasias the morphology of the tooth is at times affected. This is usually manifest by a deepening of the fissures and a loss of normal enamel contour. Per cent of dosage: 600 p.p.m.

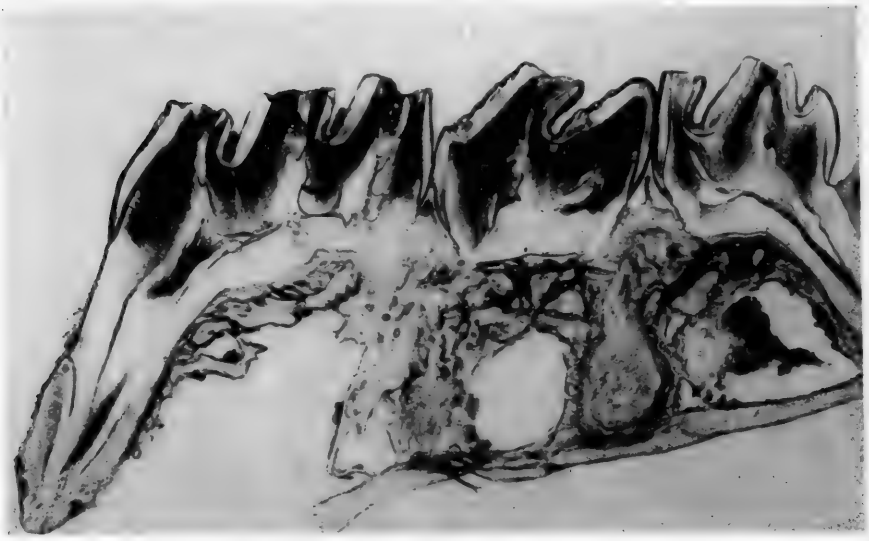

FIG. 1. Longitudinal ground section through mandibular molars of a 38-day old control animal. Note enamel-free cusp tips. (20 ×)

FIG. 2. Normal rat molars. Opposite mandibular jaw segment from rat illustrated in *fig.* 1. (10 ×)

FIG. 3. Mild mottling of rat molars. (10 ×)

FIG. 4. Moderately severe mottling of rat molars. The enamel is rough and pitted. (Third molar was lost from its follicle in the process of cleaning the soft tissue from bone.) (10 ×)

fluorine fed as potassium fluoride daily to the mother during pregnancy and lactation; young, 450 p.p.m., 1 to 30 days of age.

Microscopic findings. The details of the microscopic changes occurring in the incisor teeth of rats receiving fluorine has been fully described by Schour and Smith (1935). Except that these studies are concerned with teeth of the fixed dentition rather than those of persistent growth in this investigation, these observations are essentially in agreement with those of other workers. It would be unnecessary, therefore, to repeat the findings. It would not be ambiguous,

FIG. 5. Severe mottling of rat molars. Note excess chipping of enamel in cusp areas where occlusal stress is brought to bear. Third molar has just completed eruption. (10 ×)

however, to mention that it has been observed that fluorine given in minimal amounts necessary to produce mottling, affects the enamel of both the molars and incisors more readily than it does the dentin. The reason for this is probably that the ameloblasts of the forming tooth organ are more highly specialized than the odontoblasts. Moreover, it is probably true, as Schour and Smith observed, that the fluorine acts by exerting "... a direct local action on enamel forming cells and that the changes observed in the enamel and dentin are not produced primarily by changes in blood calcium or phosphorus or by disturbances of the parathyroids."

The production of mottling in the molars of our rats was accompanied by simultaneous changes in the incisors. Rat incisors are persistently growing teeth and consequently show changes which synchronize with changes in the molar teeth. The mottling in these teeth likewise varies in extent and severity with the amount of fluorine given. Upon discontinuation of the feeding, however, the teeth became normal as wear takes place and they are replaced by new tooth substance.

SUMMARY

1. The time periods covered by the formation of the fixed dentition of the rat is reviewed in this paper and the data checked by observing the effect of fluorine feeding upon the teeth.

2. A method is presented for the experimental production of fluorosed enamel in the molars of the rat using as a basis observations made on a group of 43 Wistar strain animals that were given graded doses of potassium fluoride for varying lengths of time immediately following birth. The results show that it is possible to produce mottling by the feeding of fluorine to young rats during the period of appositional growth and calcification of their permanent teeth. A mild type of mottling can be produced by using doses as low as 150 p.p.m. The optimal amount for mild, uniform mottling is 300 p.p.m. Concentrations varying from 350 to 550 p.p.m. produce macroscopic hypoplasia with pitting and corrosion. With high doses and extreme toxicosis, the enamel shows extensive softening and it is common for the dentin to become denuded soon after eruption of the teeth. The health of the animal likewise is affected adversely as manifest by an increased mortality in such a group. Sex did not seem to be a factor in altering the susceptibility of fluorine effects as manifest in the teeth.

3. Because mottled teeth are known to be high in fluorine content, the value of using experimentally fluorosed rat molars in studying the mechanism of action of this drug in preventing dental caries is made possible by this study.

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HISTOLOGIC STUDY ON THE EFFECTS OF FLUORINE ADMINISTERED IN DRY AND MOIST DIETS ON TEETH OF YOUNG ALBINO RAT[1]

MURRAY M. HOFFMAN,[2] CECILIA SCHUCK[3] AND WILLIAM J. FURUTA[4]

College of Dentistry, University of Illinois, Chicago, Ill. and Purdue University, Lafayette, Ind.

INTRODUCTION

The purpose of this investigation was to study the microscopic changes in the incisor and molar teeth of young growing albino rats which were subjected to daily intakes of fluorine in a dry diet, in milk, and in water. The effect of such diets upon body growth and the gross dental changes were reported in a previous study (Schuck 1939).

REVIEW OF LITERATURE

The histologic effects of a fluorine-containing diet on the incisor teeth of rats have been previously reported by Bergara (1929), Chanales (1930), Pachaly (1932), Bethke, Kick, Hall and Chase (1933) and Schour and Smith (1934). The effects of fluorine injections on the rat incisor and its surrounding tissues were initially reported by Schour and Smith (1934).

The reader is referred to Schour and Smith (1934) who have carefully summarized the findings of the various authors. Since the demonstration by the latter authors of the extreme sensitivity of the dental structures to fluorine toxicosis, the rat incisor has been employed by later investigators as a testing medium for various studies in experimental fluorosis. However, in these investigations only the outward gross changes of the incisors have been used for bases of criteria (DeEds and Thomas 1934, Ellis and Maynard 1936, Evans and Philips 1939, Hodge, Luce-Clausen and Brown 1939, Sharpless 1936 and Smith and Leverton 1934). Detailed histologic study, especially of the formative organs of the tooth, has been more or less neglected. Among the more recent literature, the dental histopathology of experimental fluorosis has been reported by Sutro (1935), Ohnell, Westin and Hjärre (1936) and Brinch (1937).

Schour and Smith (1934) and Schour and Hoffman (1939) have also shown, as a result of fluorine injections, that the incremental lines in the enamel and dentin in the rat incisor are indicative of zones of daily growth, 16 microns per day. Both the enamel and the dentin show a pair of light (hypo) and dark (hyper) in-

[1] Received for publication January 15, 1942.

[2] Department of Histology, College of Dentistry, University of Illinois.

[3] Department of Foods and Nutrition, Purdue University, with the technical assistance of Evelyn Krause.

[4] Research Fellow, American College of Dentists.

cremental layers for each injection of fluorine. Chronic fluorosis creates daily accentuations which also average 16 microns per day.

METHODS AND MATERIALS

Thirty-three albino rats were weaned at 21–24 days of age and placed on the several diets (Table I). Of these, 10 were utilized as basal diet controls. The others were given the basal diets supplemented with 0.05 per cent NaF (Table I). All the rats, with the exception of those indicated in groups D and E which failed to survive the experimental period, were sacrificed at 41 days of age. The heads were split midsagittally and fixed in 10 per cent formalin. The ani-

TABLE I

Average daily food and sodium fluoride intake and weight increment during experimental period of 20 days

Groups	A	B	C	D	E
Type of diet	Control—Sherman's Diet B	Control—Evap. Min. milk	Sherman's Diet B + 0.05 per cent NaF	Min. Evap. milk + 0.05 per cent NaF*	Sherman's Diet B + 0.05 per cent NaF in drinking water**
Number and sex of rats	2 ♂ 3 ♀	2 ♂ 3 ♀	3 ♂ 5 ♀	4 ♂ 5 ♀	3 ♂ 3 ♀
Avg. daily food intake, gms.	9.1	21.4	8.0	9.5	4.5
Avg. daily NaF intake, mg.	—	—	4.00	4.75	4.75
Avg. weight increment for Exp. Period, gms.	77.0	72.5	57.3	14.5	7.1

* Three of this group failed to survive the experimental period. The figures represent averages for the 6 surviving animals. The rats that died before the end of the experiment had severe diarrheas which probably were not the result of the toxicity of the fluoride.

** All but 2 of this group died in 7 to 8 days. The food and sodium fluoride intake and the weight increment for the 2 animals that survived the experimental period are in close agreement. Averages of these figures are given.

mals were bred and carried through the stage of sacrifice and fixation at the laboratories of one of us (C.S.).

Roentgenograms. Each half head was x-rayed with a standard dental xray machine of 50,000 volts, 10 milliamperes. The half head was placed in the film, cut surface downward, and the exposure was 6 seconds with the base of the cone 9 inches from the film.

Ground Sections. Ground longitudinal sections of the upper and transverse sections of the lower incisors respectively, and anteroposterior midsagittal sections of the lower molar segment of the left half of each head were prepared according to the method of Hoffman and Schour (1940).

Histologic Technique. The upper incisor and upper molar segment of the right half of each head were decalcified in 5 per cent nitric acid for 24 hours; dehydrated in alcohols and embedded in celloidin. Midsagittal sections, 10–15

microns thick, of the incisors and molars were sectioned with a sliding microtome, stained with hematoxylin and eosin, dehydrated, cleared and finally mounted under cover slips in gum damar.

The roentgenograms, pigment studies, and the histologic technique and observations were carried out at the laboratory of the other 2 authors (M.M.H. and W.J.F.).

Method of Study. Observations were made to determine the morphologic alterations in the enamel, dentin, cementum, pulp, alveolar bone and their approximating tissues. Measurements of the incremental lines of the ground and decalcified sections in the enamel and dentin were made with an eyepiece filar micrometer which was calibrated from a standard micrometer stage. Measurements were made in microns and were accurate to the second decimal place. Tangentially cut sections were not used for measurements.

FINDINGS

Gross. The gross findings in this experiment (Table I) were essentially the same as those reported by Schuck (1939). The results showed that those animals which had a daily fluorine intake of 0.05 per cent in water were the most severely affected, while those animals which received a daily fluorine intake of 0.05 per cent in the dry diet were least affected. The toxicity thus increased in the order of dry diet, evaporated milk and water.

Enamel Pigment. The changes in pigmentation of the enamel surface of the incisors appeared to be similar in the water and evaporated-milk groups (Table II). When the pigment was completely absent, the change was classified as most severe. The presence of accentuated cross-striations was allotted a disturbance value less than total absence of pigment. Those animals in the water and milk groups which survived the full experimental period (20 days) showed total absence of pigment. The animals which survived 7 to 8 days following the beginning of the experiment were limited to the water group and showed pigment changes which were manifested by accentuated cross-striations.

Roentgenographic. No significant roentgenographic changes were found in skulls of the rats given the fluorine-containing diet.

Histologic Findings in Ground Sections:

Incisors. Both the transverse and longitudinal ground sections of the incisors showed zonal accentuations of the daily incremental lines in the experimental animals (*fig. 1*). The control animals showed daily incremental accentuations, though the texture of the dentin was apparently normal and the lines were not so sharp as in the experimental group. Assessment of the sum total of the incremental zones in the dry diet and evaporated milk groups showed a daily appositional rate of approximately 16 microns in the enamel and dentin of the incisors. In sections which were cut within 1 to 2 mm. anterior to the mental foramen, it was possible to demarcate preëxperimental from experimental zones. There were as many accentuations of incremental lines as the number of survival days (*fig. 1*). Interglobular dentin was present in all experimental animals

TABLE II

Dietary History of Animals and Summary of Results

DIET GROUPS	PIGMENT, INCISORS	GROUND SECTIONS		DECALCIFIED SECTIONS, STAINED WITH HEMATOXYLIN AND EOSIN	
A. Control: 1) Sherman B 2) Min. Evap. Milk 5 ♂ 5 ♀ = 10	Normal	Incisors: Enamel—Normal Dentin—Normal	Molars: Enamel—Normal Dentin—Normal	Incisors: Enamel Organ — Normal Dentin, Pulp — Normal Bone—Normal	Molars: Dentin, Pulp, Bone —Normal
B. Sherman B + 0.05 per cent NaF. 3 ♂ 5 ♀ = 8	Partial loss of pigment	Incisors: Enamel — Accen. Incr. Lines Dentin — Accen. Incr. Lines Globulation	Molars: Dentin — Globulation Accen. Incr. Lines	Incisors: Enamel Organ—Ameloblastic Degeneration Amorphous Calcified Deposition Cyst Formation Accelerated Ameloblast Regression Persistence of Matrix toward Incisal Dentin — Poor Calcification; Globulation Accentuated Incr. Lines Wide Predentin Pulp—Normal Bone—Osteoid Border	Molars: Dentin — Globulation Pulp—Normal C Bone—Osteoid

C. Min. Evap. Milk + 0.05 per cent NaF. 4 ♂ 5 ♀ = 9 †	Partial loss of pigment	Incisors: Enamel — Accen. Incr. Lines Dentin — Globulation	Molars: Dentin — Globulation Accen. Incr. Lines	Incisors: Enamel Organ—Ameloblastic Degeneration Amorphous Calcified Deposition Cyst Formation Accelerated Ameloblast Regression Persistence of Matrix toward Incisal Dentin — Poor Calcification; Globulation Wide Predentin Pulp—Normal Bone—Osteoid Border	Molars: Dentin — Globulation Pulp—Normal Bone—Osteoid
D. Sherman B + 0.05 per cent NaF in water 3 ♂ 3 ♀ = 6 *	Complete loss of pigment	Incisors: Enamel — Accen. Incr. Lines Dentin — Globulation	Molars: Dentin — Globulation Accen. Incr. Lines	Incisors: Enamel Organ — Ameloblastic Degeneration Amorphous Calcified Deposition Cyst Formation Accelerated Ameloblast Regression Persistence of Matrix toward Incisal Dentin — Poor Calcification; Globulation Wide Predentin Pulp — Slight Localized Degeneration of Odontoblasts Bone—Osteoid Border	Molars: Dentin — Globulation Pulp — Localized Degeneration of Odontoblasts Bone—Osteoid Retarded Eruption of 3rd Molar

† Three animals of this group died prematurely. Av. Survival Period 18.1 days.

* Four animals of this group died prematurely. Av. Survival Period 11.5 days.

and showed an increase in severity in the order from dry diet to the water group. The latter was so severe that it was impossible to measure the incremental zones (*fig. 2*).

Molars. The molars of the experimental animals in the dry diet and milk groups showed accentuations of the incremental lines in the dentin which was formed during the experimental period. The periodicity of the effects confirms the findings on the rat molars which were given injections of alizarine Red S (Hoffman and Schour 1940). It is interesting that the initial effects in the dentin of the experimental animals were located in a position which was regularly demarcated by a single sharp line in the controls. This line in the controls and the initial accentuations in the first molars of the experimental animals was located in the crown dentin approximately two-fifths of the distance from the pulpal wall to the dentino-enamel junction. The dentin preceding this line was homogeneous and well calcified. It was interglobular and not so well calcified from this line to the pulp (*fig. 3*). This line evidently demarcates the pre- from the post-weaning period. In the third molars this increment was concomitant with the period of formation of the cemento-enamel junction. In the normal third molar of the rat, the crown formation is complete by the 21st–23rd day. Thus the effects of fluorine on the molar teeth were found to be immediate. The accentuations of the incremental lines in the experimental zones in the molar teeth of the water group were obliterated by a severe interglobular dentin. In the other groups the increments showed a distinct accentuation from the earliest to the latest manifestations.

Histologic Findings in the Decalcified Sections:

A. *Control Animals.* The histologic picture of all the control animals in the several dietary groups shown in Table I were essentially similar.

1. *Incisors.*

Enamel Epithelium. The amelogenic organ consists of an uninterrupted layer of columnar cells extending apicoincisally. In young animals the typical columnar shape of the cells is found extending to the level of the alveolar crest bone. From the latter point incisally, atrophic remnants, the united or reduced enamel epithelium, are found. This is the normal course of physiologic retrogression of the ameloblastic cells. A layer of connective tissue (labial alveolar periosteum) bearing the nutrient vessels to the outer enamel epithelium lies between the enamel organ and the labial alveolar bone.

The organic enamel matrix which stains faintly with hematoxylin persists approximately to the beginning of the middle third of the tooth. Fully calcified enamel which formerly occupied the remaining two-thirds is lost during the process of decalcification.

Dentin and Pulp Tissue. The dentin of the entire tooth is well calcified, as evidenced by homogeneous staining with the hematoxylin stain. Some interglobular texture was present, but this was considered within normal range. The incremental lines of rhythmic calcification are very faint. The predentin con-

sists of a narrow and regular eosinophilic strip (15 to 22 microns) existing between the mature dentin and the odontoblastic layer.

The odontoblasts lie in contact with the pulpal border of the predentin on the labial and lingual sides. The pulp consists of connective-tissue cells with stellate-shaped protoplasmic processes, containing vessels and nerves.

2. *Molars.* The dentin of the molar teeth is well calcified. Predentin is narrow in width and is bordered on its pulpal side by normal odontoblasts. The calcified enamel covering the crown portion of all three molars has been lost during preparation.

B. *Sherman B Diet + 0.05 per cent Sodium Fluoride.*

1. *Incisors.* The ameloblasts degenerate in small groups, especially in the region of the basal third. In these areas, amorphous calcified masses which stain heavily with hematoxylin occur between the degenerated cells and the outer enamel epithelium (*fig. 4*). In other instances a cystic formation is encountered in which the remnants of the degenerated ameloblasts can still be found in the lumen (*fig. 4*). The cyst wall appears to be formed as a result of active cell proliferation of the outer enamel epithelium. Cysts are also found more incisally at the level of the alveolar crest in the reduced enamel epithelium. The cystic development progresses from the basal to the incisal end. In most animals of this group, the entire ameloblastic layer of the incisal two thirds showed acceleration of the physiologic regressive changes. The organic enamel matrix persists well beyond the level of the alveolar crest, and in most instances was found extending up to the incisal edge.

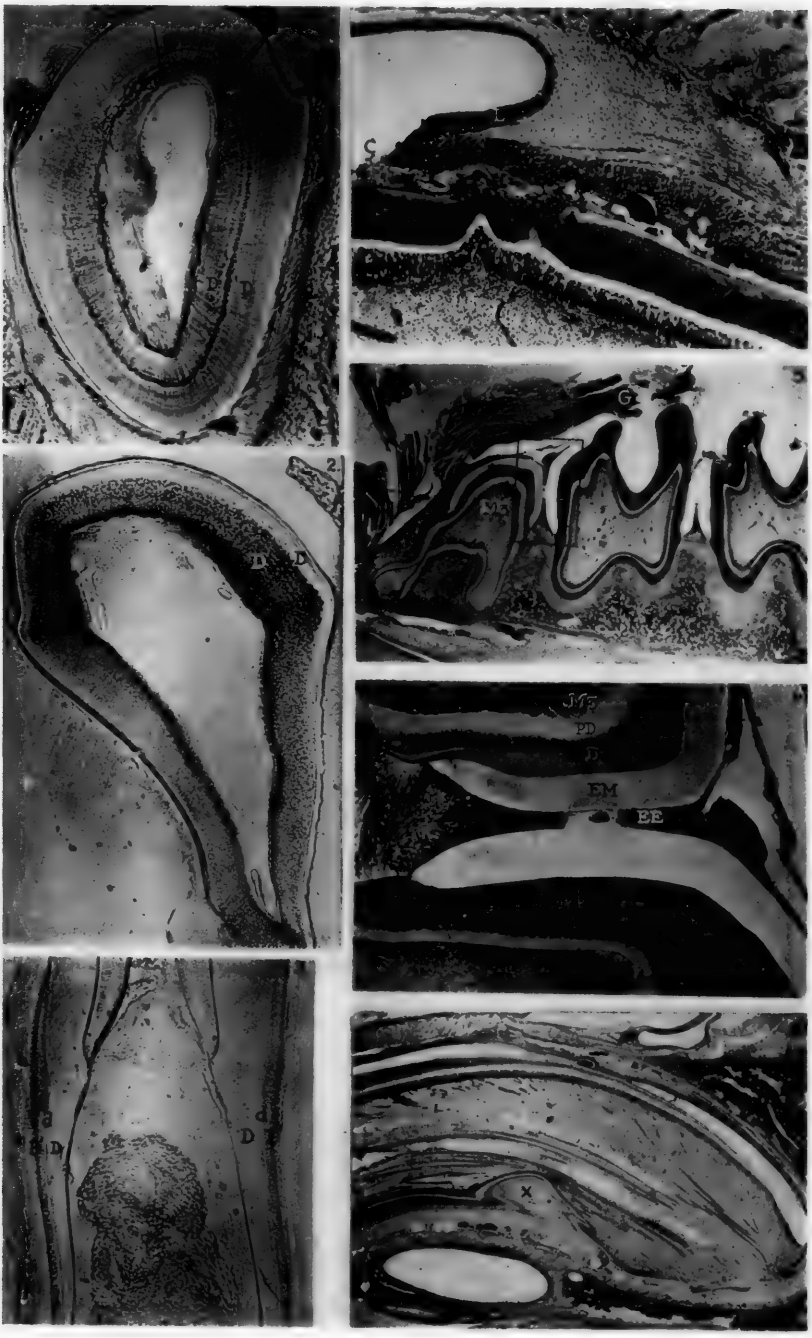
Dentin. The dentin shows marked globulation on both the labial and lingual sides, indicative of poor and irregular calcification. The incremental lines are accentuated, each pair consisting of a dark hypercalcified and a light hypocalcified band. These bands are especially numerous and prominent in the lingual dentin. The predentin, in contrast to the teeth of the controls, is abnormally wide and irregular, being approximately 2½ times the normal width (40–60 microns). Its dentinal border presents a saw toothed effect on account of the extreme globular calcification of the dentin. The odontoblasts and the pulp tissue remain essentially normal.

2. *Molars.* The portion of dentin formed during the experimental period is poorly calcified and is extremely globular. Predentin is wider than that of the normal control animals. The odontoblasts and the pulp tissue appear normal. The third molars, which erupt normally on about the 35th day (Hoffman and Schour 1940) were still submerged. The organic enamel matrix persists in its entirety (*figs. 5* and *6*). The alveolar bone around the incisor and molar teeth shows a considerable amount of eosin-staining osteoid border.

C. *Mineralized Evaporated Milk + 0.05 per cent Sodium Fluoride.*

1. Incisors.

Enamel Epithelium. The ameloblasts show degeneration, especially in the apical third of the enamel organ. Basophilic amorphous calcified masses are



FIGS 1–7

again found in these areas between the degenerated cells and the outer enamel epithelium. Cyst formation again occurs in most of the animals of this group. In some animals, in the middle third of the tooth groups of ameloblasts separate from the organic matrix and the space between them appears to have been filled with fluid. Again as in the previous group, the incisal two-thirds of the ameloblastic layer shows accelerated retrogressive changes. The organic enamel matrix persists far into the incisal extremity. It is interesting to find that the alveolar bone encroaches close upon the outer enamel epithelium so that the ameloblasts internal to this point appear to be shortened in length as a result of this pressure.

Dentin. Dentin again shows a marked globular texture. Incremental lines are very prominent, with attending hyper- and hypocalcified zones. Predentin is abnormally wide and irregular. The odontoblasts and the pulp tissue remain normal. In these animals, a large invagination of connective tissue of the periodontal membrane is found to extend into the dentin on the lingual side near the apical end (*fig. 7*).

2. *Molars.* The dentin formed during the experimental period is very globular and poorly calcified. Predentin again is abnormally wide and irregular. The

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FIG. 1. Photomicrograph of a ground cross-section of a lower incisor of 41 day old rat fed Sherman B diet with 0.05 per cent sodium fluoride for 18 days. Experimental dentin (D′) is very globular, yet the accentuated incremental lines are discernible. D, Normal dentin. Orig. mag. ×105.

FIG. 2. Photomicrograph of lower incisor of rat given water containing sodium fluoride and showing severe disturbance in dentin calcification (D′). D, well-calcified pre-experimental dentin. Decrease of width in the mesiodistal direction has occurred in the lingual half. Compare with *fig. 1*. Orig. mag. ×105.

FIG. 3. Photomicrograph of ground section of lower first and second molars of a 41-day-old rat showing root portions of 2 teeth. Animal was given Sherman B diet + 0.05 per cent NaF from the 23rd day of life to death (41 days). Note character of preexperimental, D, and postexperimental dentin, D′. The two day interval between weaning (21 days) and the beginning of the experimental diet (23 days) is demarcated by 2 sharp bands, d. Orig. mag. ×105.

FIG. 4. Photomicrograph of a midsagittal section of the upper incisor of a 41-day-old rat showing the labial mid-third. The animal was given Sherman B diet with 0.05 per cent sodium fluoride for 20 days. Note: Localized ameloblastic disturbance in the basal region, A, and the amorphous basophilic mass formed in the enamel epithelium at B. Cystic degeneration at C has resulted in outflowing of the dentin into the area normally occupied by enamel. Epithelial lining of cyst (L) is continuous with remnants of the outer enamel epithelium. Orig. mag. ×132.

FIG. 5. Photomicrograph of longitudinal section of the lower jaw of rat, 41 days old, fed Sherman B diet with 0.05 per cent sodium fluoride showing unerupted third molar (M_3). The gingival epithelium (G) covers part of the cusp of second molar. Orig. mag. ×22.

FIG. 6. Photomicrograph of a higher magnification of squared area in Fig. 5. The crown of unerupted third molar (M_3) shows persistence of enamel matrix (EM), poorly calcified dentin (D) and wide predentin (PD). G, gingival epithelium; EE, reduced enamel epithelium covering the enamel matrix. Orig. mag. ×95.

FIG. 7. Photomicrograph of midsagittal section of upper incisor of rat 41 days old fed mineralized evaporated milk with 0.05 per cent sodium fluoride showing invagination of lingual periodontal connective tissue into lingual dentin and pulp at "X". Orig. mag. ×23.5.

pulp tissue remains normal. In the animals surviving the full experimental period, the third molars were still submerged. The organic enamel matrix again persists. Cystic degeneration of the reduced enamel epithelium near the cemento-enamel junction was noted. The alveolar bone surrounding the incisor teeth and the molars shows similar pictures of abnormal osteoid borders.

D. *Sherman B + Water Containing 0.05 per cent Sodium Fluoride.*

1. *Incisor.* The greater portion of the ameloblasts in the basal third shows degenerative changes as a result of the dietary disturbance. The degeneration of the enamel organ described in the two previous groups is much more severe in this group. In an animal which survived the full experimental period of 20 days, normal enamel formation seems to have been abated in the basal portion as evidenced by poor texture of the matrix and ameloblastic collapse. These cells are atypical for this region in that they simulate the ameloblasts found in the more incisal portion of the control animals. The organic enamel matrix persists well into the incisal third of the tooth.

Dentin. Dentin is very poorly calcified and globulation is severe. Incremental striations are pronounced. In the animals surviving the full experimental period, occasional vascular inclusions are found in the incisal portion of the dentin. The predentin is abnormally wide, being approximately 2½ times greater than the normal width. Large cyst-like spaces bordered by concentration of cells of the pulp tissue are found within the pulp. Invaginating folds in the dentin, both on the labial and lingual aspects, occur in the newly formed predentin at the apical extremity of the pulp.

2. *Molars.* The experimental dentin of the molar teeth is very poorly calcified and shows severe globulation. Predentin again is markedly irregular. In the coronal portion of the pulp, in contrast to the previous group, the dentin-forming cells of the entire pulp appear to be degenerating. A higher magnification of such areas shows the disintegration of the cytoplasm with formation of numerous minute vacuoles. The remaining nuclei are round and pyknotic. Excessive cementoid occurs around the roots of the molars. Retarded eruption with persistence of the organic enamel matrix again occurs in the third molars of animals surviving the full experimental period. The alveolar bone of the incisors and the molars has an excessive amount of eosin staining osteoid.

DISCUSSION

A. Correlation with Results of Previous Investigators

Despite the short experimental period of these animals, the general pathologic changes conformed to those found by Schour and Smith (1934) and other previous investigators. In the enamel organ, enamel hypoplasia occurred as a result of ameloblastic degeneration. Grossly, the enamel surface showed the characteristic "fluorine ring," and in more severe cases a complete absence of pigment. The appearance of the incremental lines in the dentin was accentuated but they maintained their regularity in width (approximately 16 microns). Calcification was poor, as shown by marked globulation. Some globulation was also

found, although to a less extent, in the dentin of the control animals. This points toward a possible inadequacy of the so-called "optimum diets," as well as the extreme sensitivity of the dental tissues to minute dietary disturbances.

Schour and Smith (1934) found retarded rate of eruption of the incisors in fluorosis. The retardation of the eruption shown in this experiment on the molars augments the findings on the incisors.

On account of the short duration, the abnormalities were confined primarily to the formative tissues. The severity of the changes in our findings may be due to the fact that the experimental period was limited to what is normally an optimum and maximum period of growth in the rat.

The dentin changes of the incisors are interesting in that the disturbances were produced in spite of normal-appearing odontoblasts. This suggests the importance of factors other than odontoblastic activity that play a rôle in the calcification of the dentin.

B. *Relation to Gross Findings*

Both the pigment changes on the enamel surface and the histologic changes, especially those found in the ground sections, correlate with the gross findings of Schuck (1939) in regard to the relative severity of toxicosis with the three types of diets. Had all the animals in the fluorine-added-water groups lived the full length of the experiment, the tissue changes would have been much more accentuated. The difference in severity between the milk group and the dry diet group was very slight from the histologic standpoint.

Since the basic diets were adequately controlled, and the average fluorine intake per animal in each group was approximately equal, the explanation for the variation in toxicity probably lies in the difference of absorption of the mineral in the gastro-intestinal tract (Schuck 1939).

C. *Mineral Metabolism*

The rachitic-like changes found in the dentin and the presence of excessive osteoid in the alveolar bone of these rats necessitates a discussion on the correlation of calcium-phosphorus metabolism, vitamin D and fluorosis. Since the controls did not show abnormalities, the experimental changes can be ascribed to the dietary fluorine. Probably the basic diet was just adequate in calcium and phosphorus for a rapidly growing animal, but with the addition of fluorine a disturbance in the physiologic economy resulted. The mode by which fluorine exerts its effect in this instance is a matter of conjecture. Changes in calcium-phosphorus metabolism have been found only in cases of rats having a higher level of fluorine intake and longer experimental duration than ours (Lantz and Smith 1934, McClure and Mitchell 1931). Hauck, Steenbock and Parsons (1933) reported decreased total ash content of bones and teeth when fluorine was added to a diet containing a "normal" amount of calcium. Also the effectiveness of vitamin D was decreased in fluorosis (Morgareidge and Finn 1940). Phillips (1933) found a similar effect of fluorine on the efficiency of vitamin C in experimental therapy of scorbutic guinea-pigs.

The occurrence of excess osteoid in chronic experimental fluorosis was reported by Sutro (1935), Ö'Hnell, Westin and Hyärre (1936), Brinch (1937), Rohlm (1937) and Kellner (1939). A slight amount of osteoid was found in our controls. This is physiologic in young, rapidly growing rats.

D. *Significance of Molar Findings*

Apposition. The findings in the molars clearly demonstrated that the effects of fluorine toxicosis did not alter the rate or periodicity of dentin apposition. In both the ground and decalcified sections the daily lines, which were accentuated as a result of fluorine administration, composed an experimental zone which was correlated with the period of the experiment. For each day of the duration of the experiment there was an accentuated increment. The distances between the increments were not equal, nor was the rate constant. The rates followed a gradient which coincided with the rates and gradients in normal animals of comparable age (Hoffman and Schour 1940).

Eruption. Despite the normal rates of apposition there was a retardation in the time at which the molars appeared in the oral cavity. This was especially evident in the third molars. In the normal animal, the third molar appears in the oral cavity on the 35th postnatal day (Hoffman and Schour 1940). In our experimental animals which survived the full period of the experiment (41st day) the third molar was still unerupted.

Enamel. Concomitant with the retarded eruption was the persistence of the organic enamel matrix and the enamel organ in the third molar at the 41st day. This finding parallels the picture of poor dentin calcification. Since the initial increment of disturbance occurred at approximately the cemento-enamel junction in the third molars (21st day) we should expect the greater part of the organic enamel matrix to be complete in apposition and calcification. Apposition was near completion; but, as evidenced by the persistence of the organic matrix, the calcification process was not complete. Several possibilities regarding the periodicity and process of enamel calcification present themselves as a result of these findings: 1. That enamel calcification occurs en masse (Chase 1927, Diamond and Weinmann 1940) may find further substantiation here. The calcium-phosphorus physiologic economy was affected in the enamel as well as the dentin; and, if mass calcification occurs in the enamel, the "trigger point" for the initiation of the process just fell short of being started by a day or two. 2. If enamel, like dentin, calcifies by incremental calcification—that is, an increment of matrix calcifies before a subsequent increment of matrix is deposited—then it is possible that the quantitative content of the inorganic salts is present in the organic enamel matrix; but the processes of crystallization (ageing) was kept from completion by the inclusion of the fluorine. Fluorine is known to interfere with the phosphatase mechanism (DeEds 1934). It is possible that the phosphatase mechanism, normally, is not exerted until the ameloblasts cease depositing matrix (which contains the inorganic salts) and serves to organize these salts into the pattern known as completed enamel.

By the above reasoning, the duality of the calcification process suggests itself.

That is, calcification of enamel may involve both an incremental phase (wherein the salts appear with the matrix in incremental layers) and an en masse phase (wherein the salts "crystallize" to form the hardest biologic substance, enamel, by virtue of a mechanism or an enzyme such as phosphatase).

E. *Fluorine in Relation to Dental Caries*

A brief note may be made of the relation of fluorine to treatment of dental caries. Recent experimental as well as statistical surveys of clinical material indicate the apparently beneficial rôle of fluorine as a caries preventative. However, the apparent toxicity of fluorine and the ensuing pathological changes when administered, strongly suggest that very careful investigation and rationalization of findings be borne in mind to offset the misfortune that often follows the publications on "cure-alls."

SUMMARY AND CONCLUSIONS

The gross changes in the dental structures of young rats fed fluorine-containing diets in several forms were observed. Pigment changes in the incisors as well as ground sections showed that animals receiving fluorine in water were most severely affected; in milk next, and in dry diet the least.

The histologic changes found in all the experimental groups were as follows: 1. Degeneration of ameloblasts with enamel hypoplasia and cystic formations. 2. Accentuation of appearance of the incremental lines in both dentin and enamel. The rate of incrementation remained within the normal (approximately 16 microns). 3. Rachitic-like changes in the dentin of incisor and molar teeth (interglobular dentin). 4. Occurrence of excessive osteoid in the alveolar bones. 5. Retarded eruption of third molar teeth. 6. Persistence of organic enamel matrix in the incisors and third molars, possibly associated with poor mineralization. 7. The possibilities relating to normal calcification of enamel as exemplified by the findings is discussed. 8. The findings in both normal controls and the experimental animals bear out the inadequacy of so-called "optimum diets."

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CORRELATION OF CHEMICAL AND HISTOLOGICAL INVESTIGATIONS ON DEVELOPING ENAMEL[1]

JOSEPH P. WEINMANN, M.D., GEORGE D. WESSINGER, PH.D., AND GERALD REED, PH.D.

The Foundation for Dental Research of the Chicago College of Dental Surgery, Chicago, Ill.

The aim of the present investigation is to analyze the main chemical components of developing enamel and to correlate the chemical findings with the corresponding histological appearance. Such investigations are entirely lacking. All previous information on the chemistry of calcification of the enamel is based on indirect evidence. Historically, the first method for determining the changes taking place in the developing enamel was the use of the polarizing microscope (Valentin, Hoppe, von Ebner, W. J. Schmidt, Kitchin). The majority of investigators have drawn conclusions concerning the nature of developing enamel from the staining reaction of decalcified and ground sections (Beust, Mellanby, Kitchin, Chase, Diamond and Weinmann). Applebaum (1) correlated his observations made with the Grenz ray to the findings of previous investigators. In the present study on pig tooth germs an attempt is made to define the 2 distinctly separated stages of enamel development, i.e., enamel matrix formation and calcification (8), in terms of chemical values.

METHOD AND MATERIAL

All the investigations in the present report were made on permanent tooth germs of the pig. The heads of the animals were secured from the stockyard as soon as possible after the animals had been killed. The material was kept on ice until prepared for use. One half of each jaw was used for histological study, the other half for chemical analyses.

A. Histological Method

The part of the jaw containing the tooth germ to be studied histologically was divided by means of a fine jig saw blade and was fixed for 2 days in Zenker formalin solution (modified according to Maximow). After 1 day of washing in running water roentgenograms were made. The specimens were prepared according to the routine procedure of decalcification in 5 per cent nitric acid and embedding in celloidin. Those parts of the tooth germs which correspond to the parts of the tooth germs from the other side of the jaw for chemical investigations were sectioned serially, every seventh and eighth section being saved. One series using every seventh section was stained with hematoxylin-eosin. Another series containing every eighth section was stained according to Mallory (modification of Heidenhain).

The sections of tooth germs corresponding to those used for chemical analyses were described and the descriptions correlated with the chemical findings. A report in this presentation is given only for those tooth germs on which a complete chemical analysis was made.

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B. Chemical Methods

1. *Sampling:* The samples for chemical analysis were obtained in the following way. The unerupted molars of pigs 5 to 6 months old were removed from the jaw 4 to 8 hours after the animals were slaughtered. Care was taken to obtain the teeth without injuring the soft enamel and only those teeth not damaged mechanically were used. The pulp and adhering tissues were removed from the teeth and the blood was rinsed off with water and the adhering moisture carefully wiped off with a linen towel. The samples for chemical analysis (5 to 20 mg.) were then cut from the tooth with a sharp scalpel and the location from which the samples were taken was indicated on a graph, photograph or roentgenogram of the tooth. The enamel matrix was cut through its whole thickness to the dentinoenamel junction. The dentin was always so hard that it could not be cut easily with the scalpel and there was no danger of contamination of the samples with dentin. The size of the samples of soft enamel was approximately 2 by 2 by 3 mm. The samples of hard enamel from the occlusal parts of the teeth could not always be obtained easily and were removed by a combination of cutting and breaking with the same scalpel.

2. *Determination of moisture:* As soon as possible after the teeth were removed from the jaw the samples of enamel were weighed out in a 5 ml. crucible and dried to constant weight in an oven maintained at 110°C. The loss in weight obtained in this manner was taken as a measure of the moisture content. Perhaps it should be emphasized that this may not represent the total water content of the tissue but only the "free-water"; "bound-water" is probably not driven off quantitatively at the temperature used.

3. *Determination of organic material:* Organic material was determined in either of 2 ways: (a) By ashing to constant weight, (b) By digesting with concentrated sulfuric acid and superoxol with subsequent determination of nitrogen by a modification of the Nessler method (14). Color comparisons were made with the Coleman spectrophotometer using monochromatic light of 430 mμ. The two methods yielded results which were identical within the range of experimental error.

4. *Determinations of calcium:* The ash was dissolved in 1 ml. of 1 N hydrochloric acid and diluted to volume in a 10 ml. volumetric flask. Aliquots were used for calcium estimation by the Clark-Collip modification of the Kramer-Tisdall method (6). All analyses were made in duplicate.

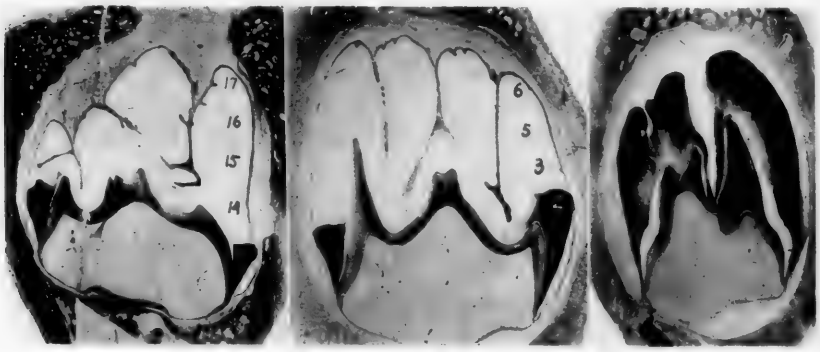
5. *Determination of phosphorus:* Aliquots of the acid solution of ash were used for phosphate estimation by the Fiske-Subarrow method (12). Color comparisons were made with the Coleman spectrophotometer using light of 830 mμ wave length. All analyses were made in duplicate.

HISTOLOGICAL FINDINGS AND QUANTITATIVE ESTIMATIONS OF THE MOISTURE, ORGANIC MATERIAL, CALCIUM AND PHOSPHOROUS CONTENT OF THE ENAMEL

Fig. 1a shows a buccolingual section of the mesial portion of a lower third molar. The development of the tooth germ in this portion has advanced to beginning dentin formation in the bifurcation. The greater part of the enamel disappeared during preparation; only in the cervical region a wedge-shaped portion of enamel escaped destruction. In this part 2 different types of lines can be seen. Striae of Retzius' meet the dentino-enamel junction at an acute angle and run almost parallel to the surface of the cusp. Schreger's lines run at a cross-relation to the former. The border between the soluble and insoluble parts of the enamel runs at almost a 90 degree angle to the long axis of the tooth and in cross-relation to the Retzius' line.

The content of moisture and organic material shows a definite gradient increasing from the occlusal area to the cervix. There is an abrupt change in

the content of organic material corresponding approximately to the border of soluble and insoluble enamel. Conversely, the calcium and phosphorus content of the samples show a gradient decreasing from the occlusal to the cervical area.



a b c

FIG. 1. Right lower third molar of pig (P III). Figures in insoluble and soluble enamel indicate approximate locations from which samples for chemical analysis were secured and which correspond to number of sample in Table I; a, mesial; b, middle; c, distal portion of tooth germ.

TABLE I

NO. OF SAMPLE	WEIGHT OF DRY SAMPLE	% MOISTURE	% ORGANIC (DRY WT.)	% Ca (DRY WT.)	% P (DRY WT.)	Ca/P RATIO	% Ca ASH	% P ASH
1	5.1	30.5	39	24.8	12.0	2.07	40.6	19.6
2	9.1	40	20	30.9	15.2	2.03	38.6	19.0
3	14.8	37	8	34.8	17.9	1.94	37.8	19.4
5	16.3	25	6.5	36.7	18.1	2.03	39.2	19.3
6	14.4	22	5.5	36.5	18.0	2.03	38.6	19.0
7	7.7	31	36	24.7	12.0	2.06	38.6	18.7
8	7.9	28	34	25.9	12.8	2.02	39.2	19.4
9	8.3	30	35	24.6	12.7	1.93	37.8	19.5
10	14.5	31.5	36	24.8	12.1	2.05	38.7	18.9
11	9.8	33.5	38	23.4	12.0	1.95	37.7	19.3
12	13.9	32.5	33	25.8	13.0	1.98	38.5	19.4
13	7.4	37	26	28.9	14.5	1.99	39.0	19.6
14	10.4	22.5	9.5	36.5	17.5	2.08	40.3	19.3
15	7.6	14.5	8	36.7	17.6	2.08	39.9	19.1
16	15.6	11	5	37.6	17.4	2.16	39.5	18.3
17	7.8	6.5	4	37.6	17.6	2.13	39.2	18.3

Fig. 1b represents a buccolingual section through the middle portion of the same tooth germ from which *Fig. 1a* was taken. This portion differs from the mesial portion in 2 respects. The part of the enamel not soluble in 5 per cent nitric acid is larger than in the mesial portion and there is no indication that dentin formation has started in the bifurcation.

The moisture and organic material show an increasing gradient from the occlusal to the cervical samples, displaying a sudden change in the organic material in the vicinity of the junction of soluble and insoluble enamel. The values for calcium and phosphorous decrease from the occlusal to the cervical region.

Fig. 1c shows a section through the distal portion of the same pig tooth from which *fig. 1a* and *1b* were taken. The appearance of this part of the tooth is essentially different from the specimens previously discussed. The entire enamel is acid insoluble. Between the labial and lingual cusps and between enamel matrix and dentin are indications of shrinkage. The striation, or Schreger's line, runs at almost a right angle to the axis of the tooth. In some areas Retzius' lines can be seen.

None of the investigated constituents shows a gradient. The variation in the content of moisture, organic material, calcium and phosphorus is slightly

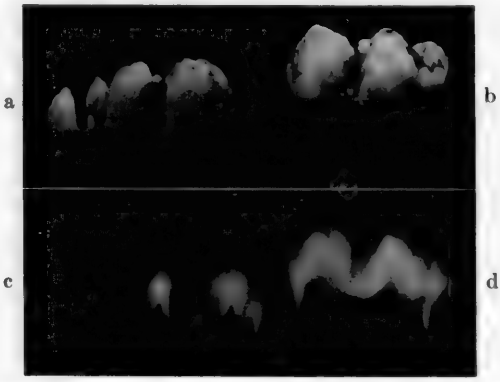


FIG. 2. Photographs and radiographs of teeth used for chemical studies. a and b—lower right third molar; c and d—lower left second molar.

greater than the expected experimental error. The average moisture content is 31.1, organic material 35.3, calcium 24.9, phosphorus 12.4.

Figs. 2a and *b* are the photograph and roentgenogram of the lower right third molar which was used for chemical investigation. The corresponding tooth germ in the same jaw was used for the section from which *figs. 3a* and *b* were taken. *Fig. 3a* shows a section through the mesial portion of this tooth germ. The greater part of the enamel is insoluble in 5 per cent nitric acid, only that enamel close to the fissure showing slight solubility. The insoluble enamel is not stained uniformly by hematoxylin, the outer layers being darker than the layer adjacent to the dentino-enamel junction, especially at the tips of the cusps. The lighter and darker stained areas are separated by a heavy Retzius' line. At some places along the course of this heavy Retzius' line the 2 layers are spread apart. The white space between enamel and dentin is an artifact. There is no indication that root formation has started in this portion of the tooth germ.

The 3 samples of the most cervical region (*6*, *7*, and *9*) show similar figures for the moisture content, organic material, calcium and phosphorus, and are very

close to the values found for the insoluble enamel illustrated in *fig. 1c*. The average moisture content is 33, organic material 32.3, calcium 24.5 and phosphorus 12.6. The sample from the occlusal area of the cusp shows a lower moisture and organic content and a higher calcium and phosphorus as compared to the sample from the cervical area. This sample was very large and may have included parts of the enamel which showed slight solubility.

Fig. 3b shows the middle portion of the same tooth germ from which *fig. 3a* was taken. The entire enamel is insoluble in 5 per cent nitric acid. There is no histological indication of enamel solubility, even at the most peripheral tips of

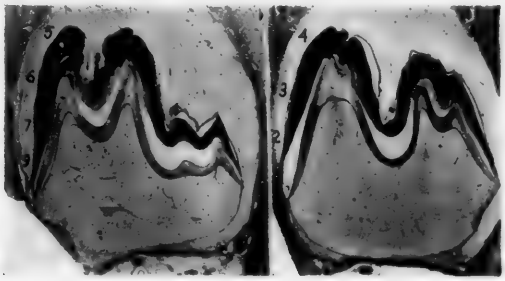


FIG. 3. Lower right third molar of pig (P1F). Figures in enamel matrix indicate approximate locations from which samples for chemical investigations were secured and which correspond to numbers of samples in Table II; a, mesial; b, middle portion of tooth germ.

TABLE II

NO. OF SAMPLE	WEIGHT OF DRY SAMPLE	% MOISTURE	% ORGANIC (DRY WT.)	% Ca (DRY WT.)	% P (DRY WT.)	Ca/P	% Ca ASH	% P ASH
2	18.9	33	34	27.2	12.6	2.16	41.7	19.2
3	12.3	30	32	26.0	13.0	2.00	38.1	19.1
4	10.8	28	27	28.3	12.9	2.20	38.8	17.6
5	22.1	25	21	29.0	14.4	2.00	36.9	18.4
6	10.75	33	34	24.5	12.5	1.93	37.3	19.3
7	11.5	32	33	24.2	12.6	1.91	36.4	19.0
9	10.2	34	30	24.9	12.7	1.96	35.8	18.3

the cusps. There is no sign that root formation has started. Retzius' and Schreger's lines are clearly seen.

The values for the various components show slight variation. The average moisture content is 30.3 per cent, organic material 31 calcium 27.1 and phosphorus 12.8 per cent.

Figs. 2c and *d* are the photograph and roentgenogram of a lower left second molar of a pig on which chemical investigations were made. The corresponding tooth germ furnished the specimens from which the photomicrographs seen in *Figs. 4a, b* and *c* were taken.

Fig. 4a shows a labiolingual section through the mesial portion of the tooth germ. The greater part of the enamel is dissolved. Wedge-shaped remnants

which were not dissolved are found on both labial and lingual sides. The border between soluble and insoluble parts of the enamel runs at a right angle to the axis of the tooth. The junction is not clear cut but shades from a dark blue to a very light blue in the hematoxylin-eosin stain. Root formation is advanced and dentin formation in the bifurcation has started. There is a definite gradient in the amount of moisture, organic material and calcium. A sharp drop in the moisture and organic material corresponds approximately to the junction between soluble and insoluble enamel.

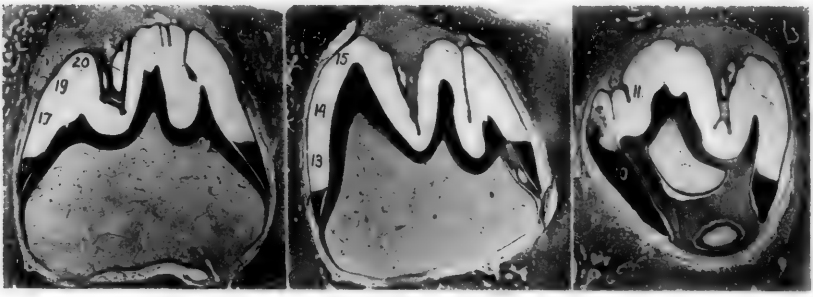


FIG. 4. Lower left second molar of pig (P2F). Figures in insoluble and soluble enamel indicate approximate locations from which samples for chemical investigation were secured and which correspond to number of samples in Table III; a, mesial; b, middle; c, distal portion of tooth germ.

TABLE III

NO. OF SAMPLE	WEIGHT OF DRY SAMPLE	% MOISTURE	% ORGANIC (DRY WT.)	% Ca (DRY WT.)	% P (DRY WT.)	Ca/P	% Ca ASH	% P ASH
10	9.1	30	33	25.7	12.1	2.11	38.3	18.1
11	19.1	15	9	34.6	16.5	2.09	37.9	18.1
12	6.8	35	29	26.2	13.4	1.96	37.1	18.9
13	11.0	21	10	35.7	16.8	2.15	39.6	18.3
14	9.9	15	8	35.1	17.0	2.07	38.1	18.4
15	15.0	16	7	36.1	17.7	2.04	38.7	19.0
16	4.9	30	22	30.3	15.3	1.98	39.1	19.7
17	22.3	17	9	35.6	17.2	2.07	39.1	18.9
19	17.3	10	7	35.1	17.7	1.99	37.8	19.0
20	9.0	10	6.5	35.8	16.6	2.16	38.3	17.7

Fig. 4b shows a labiolingual section through the middle portion of the same tooth germ from which *fig. 4a* was taken. Again the greater part of the enamel is dissolved, only a small remnant being left in the cervical region. Root formation has already started as is indicated by the deposition of cementum on the dentin in increasing thickness from the cemento-enamel junction toward the apex. The bifurcation is determined by epithelium. Dentin formation has not started yet in this area. All investigated constituents show a gradient, increasing from occlusal to cervical in the values of moisture and organic material

and decreasing in this direction in the values of calcium and phosphorus. The percentage of organic material shows an abrupt change approximately corresponding to the border between soluble and insoluble enamel.

Fig. 4c shows a labio-lingual section through the most distal portion of the same tooth germ from which *figs. 4a* and *b* were taken. That root formation has progressed is indicated by the deposition of cementum on the surface of the dentin. The enamel on the occlusal half of the crown is soluble, whereas, the enamel on the cervical half is insoluble. This insolubility is more extensive on the lingual side. The border between soluble and insoluble enamel is not sharp but shades. The junction runs on the labial side almost at a right angle to the long axis of the tooth. On the lingual side there is a similar relationship as far as the deep blue stained part of the enamel is concerned. The border between the lighter stained enamel and the completely soluble enamel is irregular.

One sample for chemical investigation was taken from the cervical part of the enamel. The values nearly coincide with those found for the insoluble part of the enamel in the other portion of this tooth. Comparing the figures of organic material taken from the cervical region of the mesial, middle and distal portions of a tooth germ a gradient, decreasing from distal to mesial, is evident. It also will be noted that the calcium and phosphorus content in the cervical region of the distal cusp is lower than that for the corresponding area of the middle and mesial cusps. The second sample was taken from the occlusal $\frac{1}{3}$ of the enamel omitting the most occlusal part. The values of the investigated constituents correspond to those found in areas 14 and 17 of the middle and distal portions of the same tooth.

DISCUSSION

Developmental Gradients. It is commonly accepted that enamel is secreted by the ameloblasts. The ameloblasts become differentiated and begin their function of enamel formation after the first layer of dentin has been formed at the tip of the cusp or at the incisal edge. As the dentin formation proceeds cervically, new groups of ameloblasts gradually and rhythmically become differentiated for the production of enamel. The gradually progressing differentiation of the ameloblasts from occlusal to cervical makes it possible to study different stages of development in the same tooth germ (18, 9). In addition, there exists a second developmental gradient from the mesial to the distal regions (3). In all 3 tooth germs of the pig described in the present report, occlusocervical and mesiodistal gradients in the development were observed.

It is likewise universally accepted that newly-formed enamel is insoluble in weak acids, whereas mature enamel is acid-soluble (11, 7). Investigation (8) on human teeth and on the molars and incisors of the rat have shown that transformation of insoluble into soluble enamel begins at the most peripheral area incisally or occlusally. This process starts only after the entire thickness of the enamel in an insoluble form has been completed in these regions. It has been shown that the process of becoming soluble advances from occlusal to cervical and that the junction of the soluble and insoluble parts of the enamel runs in

cross-relation to the incremental lines which are already present in the insoluble form of the enamel. The specimens used in the present study corroborate these observations.

Correlation of Chemical Analyses with Histological Findings. From the correlation of the chemical analyses with the histological picture of the specimens described previously the following conclusion can be drawn: The values for the components found in the acid-insoluble part of the enamel showed the following range: Moisture, 37–28 per cent; Organic material, 38–22 per cent; Calcium, 23.4–30.3 per cent; phosphorus, 12–15.3 per cent.

The variations in the values found were not regularly distributed but were larger than the expected experimental error. The range of variation was smaller if the values taken from samples bordering the soluble enamel were left out of consideration. Although a gradient in the insoluble enamel could not be proved, such a gradient may be present. Inability to demonstrate this gradient may be attributable to technique. The figures from the cervical part of the insoluble enamel (*fig. 4* and Table III) suggest a gradient from mesial to distal.

At the junction between the soluble and the insoluble enamel there is a sharp and definite change in the chemical composition expressed in the content of moisture and organic material as well as calcium and phosphorus.

In contrast to the insoluble enamel, there is definite proof of a gradient from occlusal to cervical in the chemical composition of that portion of the enamel which is soluble in 5 per cent nitric acid. The range of values found is: Moisture, 37[2]–6.5 per cent; Organic material, 9.5–4.0 per cent; Calcium, 34.6–37.6 per cent; phosphorus, 16.5–18.1 per cent.

In every case where the histological picture revealed some of the enamel to be soluble, the gradient ran parallel to the long axis of the tooth, i.e., the amount of organic material and moisture increase from occlusal to cervical while the calcium and phosphorus decrease in this direction.

Enamel Matrix. The correlation of the histological appearance with the chemical components of the different developmental stages of enamel serves as a basis for defining and classifying insoluble enamel much more distinctly than was possible in the past, and in addition helps to explain knowledge gained by other methods of investigations. Insoluble enamel is chemically characterized by a high content of moisture and organic material, while the total calcium and phosphorus is appreciable, but falls far short of that found in a fully developed erupted tooth. The insolubility of enamel of such a composition is due to its high content of organic material. The high content of organic material is probably the reason why such enamel can be stained intravitally (13) and postmortem (17). That insoluble enamel is found to be radiolucent for Grenz rays (1) is explained by the comparatively low content of mineral salts. Present investigations showed that the calcium:phosphorus ratio is fairly constant in all specimens from different developmental stages and is well within the range of the values for the enamel of erupted human teeth (4).

[2] This maximum value is probably incorrect since the next lowest value was 25–6.5 per cent.

Although insoluble enamel may undergo some changes, the fact that it is primarily the product of a formative process and is chemically characterized by a high content of organic material and moisture, justifies its classification as enamel matrix. A sharp separation of the stage of enamel matrix from the following stage of maturation is indicated by the fact that the latter does not start before the first is completed.

Enamel Calcification. It has been taken for granted that the solubility of enamel indicates its complete maturity. This assumption is refuted by the correlation of the histological picture with the chemical analysis.

Histological investigations have shown that the transformation of insoluble into soluble enamel starts at the most occlusal area of the cusp after the formation of enamel matrix has been completed and progresses in cross relation to the incremental lines (8). Chemical analysis shows that the soluble enamel of unerupted teeth is not of uniform chemical composition. The soluble enamel in the specimens investigated was found to contain as much as 9.5 per cent organic material and as little as 34 per cent calcium. The possibility that soluble enamel may contain more organic material and less calcium than indicated by the above figures is not definitely excluded. From these threshold levels the amount of organic substance and water decreases gradually, whereas the amount of mineral salts increases. This process is always most advanced at the tip of the cusp and less evident at the cervical region. If this process is called maturation of the enamel, then it can be stated that the change of insolubility into solubility marks only a stage in the gradual maturation of enamel. The change seen in the histological picture occurs when the amount of organic material decreases to a certain percentage. This point is reached when the enamel contains between 10–20 per cent organic material; the endpoint is probably nearer the smaller figure. The maturation process goes on for some time after the stage of solubility has been reached. Even the investigated stages of soluble enamel most advanced in regard to their chemical properties fall short of the composition known for fully developed enamel of erupted teeth.

From these observations it can be concluded that what appears in the histological specimens of unerupted tooth as dissolved enamel may not be mature enamel. The histological picture reveals only that the formation of enamel matrix has been completed and that maturation has started and reached at least a certain stage. It does not reveal how far maturation has advanced. Only by chemical analysis is it possible to determine whether or not enamel is mature.

The stage during which the enamel becomes soluble is characterized by a tremendous influx of mineral salts. Therefore, it seems logical to term this process, "calcification." In order to appreciate the magnitude of the increase in mineral salts during the calcification stage, it is necessary to find how much of the mineral content is present in the enamel matrix before calcification starts.

The values of organic material and mineral content for enamel matrix and calcifying enamel given above are reported as per cent by weight. These values by weight are insufficient to answer the question because of the considerable change in density of the enamel matrix during calcification. In order to com-

pare the mineral content of the enamel matrix with that of the calcifying enamel it was necessary to consider the per cent by volume. The values of weight per volume are obtained by multiplying the per cent by weight (moist) by the density of the material.

Unfortunately, the 5 to 20 mg. samples used for the chemical analysis were too small to allow density estimations on these same samples. Therefore, larger samples (100 to 200 mg.) of enamel matrix were taken from the gingival sections of the tooth germs, corresponding in development and location to that part of the matrix which contained about 30 per cent organic material. The density of these samples was estimated with the pycnometer and found to vary between 1.6 and 1.7. The calcifying enamel obtained from the occlusal parts of the tooth germs with an estimated 5 per cent organic material showed densities from 2.6 to 2.75.

Calculation of the concentration of minerals per volume of enamel matrix shows that only about 25 per cent of the minerals of the calcifying occlusal part of the tooth are present in the uncalcified enamel matrix in the gingival parts. This calculation was based on the following values:

	I Mineral content, per cent of moist weight	II Density	III Mineral content, gr. per ml.
Enamel matrix	42	1.6	0.67
Calcifying enamel	84	2.75	2.31

It is obvious from these approximate estimations that 1.64 grams of minerals per ml. have to be added in order to transform our specimen of enamel matrix into the specimens of calcifying enamel. Still more minerals would have to be added during calcification in order to obtain fully calcified and mature enamel.

In the accumulation of mineral salts in the developing enamel, which leads finally to the formation of the hardest tissue in the body, the fact that phosphatase is present and may play a leading role has been demonstrated by previous investigators and was confirmed by our experiments.

The investigation demonstrates that during the stage of calcification not only an influx of mineral salts takes place, but, at the same time, organic material and water are removed. In order to elucidate the mechanism whereby the organic material is removed, it becomes necessary first to determine its nature. In the past the organic enamel matrix has been considered to be a protein. As previously mentioned, the organic material in the samples used in this study was determined by 2 methods, viz., ashing and by nitrogen determination. Values obtained with both methods agreed within the limits of error if the per cent of nitrogen was multiplied by the usual factor, 6.25, in order to determine the per cent of protein. This suggests that the organic material is for the most part protein. It is known that insoluble protein can be removed by proteolytic cleavage. The presence of a proteolytic enzyme was suggested by a few preliminary experiments conducted in this laboratory.

The results of the chemical analysis of the insoluble enamel explain in a simple manner previous observations obtained by different methods. The solubility of

the enamel after its formation is completed is due to the effect of the small amount of organic material. The high content of mineral salts explains why soluble enamel is radiopaque compared to insoluble enamel. The increase of mineral salts and the decrease of organic material during the calcification of enamel supports the hypothesis of W. J. Schmidt (19), who explained the simultaneous change from young to adult enamel and from positive to negative birefringence by the filling up of the spaces between the submicroscopic crystal of the young enamel with more crystals as the enamel matured.

SUMMARY

By correlating the chemical analysis with the histological appearance of developing enamel, the main chemical components of the 2 stages in enamel development, i.e., matrix formation and calcification were established.

Insoluble enamel matrix contains 37–28 per cent water, 38–22 organic material, 23.4–30.3 calcium and 12–15.3 phosphorus. The range of components is smaller if samples which border soluble enamel are left out of consideration. It is calculated that in the enamel matrix about ¼ of the amount of mineral salts found in hard enamel is present.

In contrast to the insoluble enamel, there is presented definite proof of a gradient from occlusal to cervical in the soluble enamel which runs in cross-relation to the incremental lines. The values were found to range between about 25–6.5 per cent water, 9.5–4.0 organic material, 34.6–37.6 calcium and 26.5–18.1 phosphorus.

The chemical properties of insoluble and soluble enamel corroborate the morphologically based postulate that there is a distinct differentiation between the stages of enamel matrix formation and enamel calcification, the latter stage starting only after the first has been completed.

Solubility of enamel indicates completion of the enamel matrix and shows that calcification has progressed at least to a certain point but does not mean that enamel is mature.

Calcification of enamel is characterized by a removal of moisture and organic material and an influx of mineral salts. In this process a proteolytic enzyme and phosphatase may play leading roles.

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A STUDY OF THE DEVELOPMENT OF DENTAL ENAMEL IN DOGS[1]

G. E. GLOCK,[2] H. MELLANBY,[3] M. MELLANBY,[4] M. M. MURRAY[2] AND J. THEWLIS[5]

University of London, University of Sheffield, National Institute for Medical Research (London) and National Physical Laboratories (Teddington), England

INTRODUCTION

It has been shown that in the majority of human teeth there are macroscopical and microscopical structural defects which may be associated with increased liability to caries (M. Mellanby, 1923–1937). These defects have been classed as hypoplasia ("M-hypoplasia"), but must not be confused with the gross defects ("G-hypoplasia") which were the only ones generally recognized and which may or may not be associated with increased liability to caries.

In order to ascertain why these defects (M-hypoplasia)—especially in the enamel, where caries begins—are so common, it is important to understand their histological, chemical and physical characteristics in relation to the normal, and it was primarily with this object that the present work was begun. Such a study involves an intimate knowledge of the development of enamel and of the effect of diet upon it. This knowledge we do not at present possess, and a certain amount of exploratory work must therefore be undertaken, the value of which may lie both in the actual results obtained and in the indications afforded as to the nature of future problems and the methods required for their solution.

As suitable human material for chemical, physical and histological examination was not available, the developing enamel of puppies' teeth was used. It is known that the structure, properties and liability to caries of human enamel differ from those of dogs' enamel to some extent and it is not legitimate, therefore, to conclude that the results obtained in this work are necessarily applicable to human enamel, although structural defects similar in many ways to those found in human teeth can be produced experimentally in dogs by dietetic measures (M. Mellanby, 1918–1937). It is hoped that future work may deal with this problem.

In the course of studies on the effect of diet on the development of puppies' teeth, M. Mellanby (1929) noted that enamel calcifies in two fairly well defined stages (*fig. 1*). This was also seen in developing human teeth (1934). The

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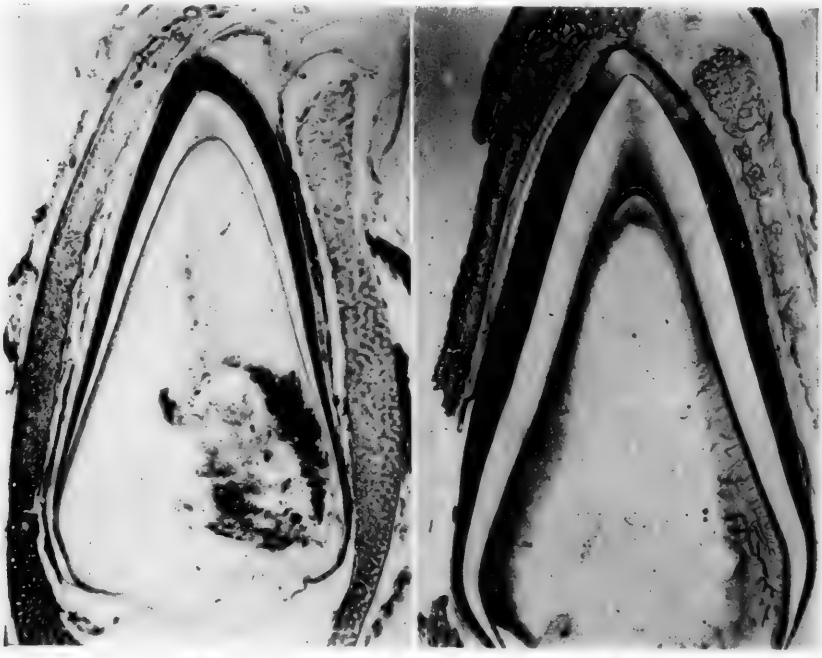
[2] Physiology Department, Bedford College (University of London), N.W.1.

[3] Field Laboratories, Lodge Moor, University of Sheffield.

[4] Nutrition Building, National Institute for Medical Research, Mill Hill, London, N.W.7.

[5] National Physical Laboratory, Teddington, Middlesex.

first stage begins at the cusps and consists of the deposition, layer upon layer, of easily cut, stainable material, each successive layer spreading further towards the neck of the tooth until the full depth of the matrix is built up. In the second stage more inorganic salts are deposited in this matrix, so that it loses much of its power to take up stains and now represents the more or less fully calcified tissue. This second stage of calcification appears, in contrast to the first, to take place comparatively rapidly throughout the depth of the enamel in a given region, first at the tip of the cusps, and later at the sides and finally



a b

FIG. 1. Photomicrographs (× 8) of carmine stained ground sections of left lower carnassials of normal puppies showing the two stages of calcification (see p. 2).
(a) 6 weeks old. All enamel deeply stained (stage I only).
(b) 14 weeks old. Enamel at the tip unstained (stage II).

in the cervical region. The first stage in the cervical region is not necessarily complete when the second stage begins in the cusps. When the matrix alone has been deposited (stage I) it is easily retained in decalcified sections, whereas when the calcification has progressed to stage II (*fig. 1*), this is difficult and Malleson's or some other special technique has to be adopted, except sometimes in hypoplastic teeth. It was also shown that when a deficient diet affected the first stage of calcification the thickness of the enamel in the fully developed tooth tended to be less than normal in some regions, whereas when the second stage was affected the enamel when complete might be of normal thickness and

yet be imperfectly calcified. In many cases both stages were found to be affected.

Beust (1928), studying the development of pig molars, considered that enamel is not deposited in finished layers, but is first deposited as a keratin-like substance containing only a small amount of inorganic material; at this stage it is easily removed by the finger nail. Calcification of this matrix then takes place. These 2 stages are to be compared with stages I and II of Mellanby.

Stages in the development of human enamel have been studied by several workers. Von Ebner (1906), using chiefly ground sections, concluded, on the basis of staining reaction and birefringence, that 4 stages of enamel development —primary, young, transitional and mature or hard—could be recognised in the transition from the soft embryonic state to its final hard condition. The findings of von Ebner have been amply confirmed by Chase (1935), Kitchin (1933) and particularly by Schmidt and his coworkers.[6]

Chase (1935) correlated the staining reactions of enamel at different stages of development with acid solubility. He found the process of maturation or calcification in human enamel to be complete approximately 3 months after the initial deposition of the enamel prisms by the ameloblasts. If calcification was not completed in this time, an incomplete stage, usually the transitional stage, was found to persist. This corresponds, presumably, to some form of M-hypoplasia.

Schmidt (1923/35, 1924, 1934), using the polarised microscope, showed that although von Ebner's observations were correct, his explanations were wrong. Von Ebner believed the phenomenon of birefringence exhibited by enamel was due to mechanical strain and that the enamel itself was essentially non-crystalline. It has, however, been shown conclusively by x-ray examination and by the polarising microscope that enamel is, in fact, a crystalline material (for references see Thewlis (1940)).

By means of the polarising microscope, Harders-Steinhäuser (1938) has shown that the first portion of the enamel matrix to be calcified is a narrow strip along the amelo-dentinal junction. "Calcification then proceeds quickly at the tip of the tooth and the completely calcified enamel soon extends across the whole depth. Towards the base the calcification proceeds at a considerably reduced rate." A similar conclusion has been arrived at independently by Applebaum (1938), who examined developing tooth sections radiographically.

It appears that some qualitative but not quantitative work has been done on the changes taking place in developing enamel. The results to be reported here are a preliminary attempt to study these changes by quantitative chemical and x-ray analysis of enamel at different stages of development and under different dietetic conditions, and to correlate these differences with one another and with histological findings.

Four series of experiments were performed. In the first, the method of enamel

[6] Diamond, M., and Weinmann, J. P. (*Enamel of Human Teeth* (New York, Columbia Univ. Press: 1940)) and Weinmann, J. P., Wessinger, G. D. and Reed, G., *J. D. Res.* **21**: 171, 1942, have recently contributed to this field of investigation (Ed.).

removal was tested and an attempt made to see how far any of the 3 grades of enamel, which will be discussed later, corresponds to either of the 2 stages of Mellanby. In the second, a study was made of the developing enamel of puppies of different ages and a comparison made of the chemical composition and x-ray structure of the good enamel formed when cod liver oil is included in the diet with the defective (M-hypoplastic) enamel produced when the cod liver oil is replaced by a vitamin free oil, such as peanut, for a sufficiently long period. The third and fourth series were concerned with the effects of addition to the basal diet (deficient in vitamins A and D) of cod liver oil, of mammalian liver oil extracts rich in vitamin A ("Avoleum") and vitamin D_2 (formed by the irradiation of ergosterol ("Radiostol")[7]), or of calcium carbonate.

The permanent teeth of the puppies were used in each case, the ages of the puppies ranging from 11½ to 21 weeks, and the experimental diets were given for periods of 6 to 14 weeks.

EXPERIMENTAL METHODS

Diet. On admission to the laboratory, the dogs were kept in separate cages under similar hygienic conditions and were given a similar amount of the same complete and adequate diet for the short period between admission and experimental feeding. The basal experimental diet included a cereal product (in these experiments white bread), meat, separated milk powder, yeast, orange juice and sodium chloride. In addition an oil, either peanut, olive or cod liver oil, was given, the first 2 supplemented when desired by vitamin A or D_2 or by both. In litters 1, 2 and 3, constant amounts of food were given, except in the case of bread, the amount of which was raised as the animal grew older and ate more. In litter 4 an attempt was made to give a definite percentage of each ingredient so that as the quantity of food eaten increased the relative proportions of the ingredients remained the same. The basal diet was in all cases relatively low in calcium and in vitamins A and D.

Separation of Enamel. When permanent tooth-germs are removed from the jaws of puppies between 11½ and 21 weeks of age it is found that the enamel which covers the teeth exhibits various stages of hardness. Most of the teeth used in these experiments had not pierced the gingivae, though a few in the older dogs had just begun to erupt. Up to 17 weeks of age it is possible to remove all the enamel from most teeth by the simple method of scraping it off the underlying dentin with a scalpel but in older puppies this method is impracticable. The enamel in any one developing tooth can be divided roughly into grades depending on its appearance and hardness.

The general method of procedure was to kill the puppies at prearranged ages, take radiographs of the upper and lower jaws and remove all the permanent tooth germs in their sacs from one side of each jaw. Each developing tooth was then taken from its sac and kept in an atmosphere saturated with water vapour until analysed. Before removing the enamel, the outer calcified shell, consisting

[7] We are indebted to Messrs. British Drug Houses Ltd. for a free supply of Avoleum and Radiostol.

of enamel and some dentin, was separated from the soft gelatinous inner portion which was discarded. For chemical analysis it was found practicable to separate developing enamel into 3 grades, although all 3 did not necessarily occur in every tooth (*fig. 2*). Grade A enamel was characterised as being nearly white, opaque and hard; it was sufficiently brittle to be chipped from the tooth with a knife. It was thought that it would probably correspond approximately with stage II of Mellanby. Grade B enamel was yellowish in colour, slightly translucent and soft. Grade C was darker, very soft and peeled off in long flakes when scraped with a scalpel. There was a greater difference between grades A and B than between B and C.

FIG. 2. Photograph (× 6) of excised erupting lower carnassial of a 16 weeks old dog showing differences in surface appearance on which, together with differences in texture, the removal of the three grades of enamel (A, B and C) was based (see p. 5). N.B. Zone B (from which grade B enamel was removed), being yellowish in colour, appears dark in the photograph.

Except in litter 1, which was used primarily for testing the method, separation into the 3 grades, A, B and C, was adopted only for reference animals. In all the other cases the enamel was removed from 3 regions, termed I, II and III, corresponding in position to those covered by A, B and C enamel, respectively, in the reference animals. Thus, in a series of animals of different ages, regions I, II and III would consist respectively of A, B and C enamel in the youngest animal, and with increasing age more and more A enamel would be found in region II and more and more B enamel in region III. This is illustrated in *fig 3*.

Histological Methods. Stained median longitudinal sections prepared by Weil's method were made through the main cusp of the left upper first incisors and left upper and lower carnassials *in situ* for each experimental dog. For each dog of any one family, slices of about the same thickness (approximately 5

mm.) were cut and stained in bulk in carmine[8] for 3 to 4 weeks before the ground sections were prepared, so that for each family a roughly comparable series of sections was obtained. In addition, in some cases decalcified sections of the carnassials were prepared and stained with haematoxylin and eosin and with Mallory's connective tissue stain.

Chemical Methods. The chemical methods employed were those adopted in all the previous chemical work on teeth by Murray and co-workers. Calcium was estimated by the method of Clark and Collip (1925), magnesium by the hydroxyquinoline method of Greenberg and Mackey (1932), phosphorus by the method of Fiske and Subbarow (1925) and CO_2 by using a modified Van Slyke apparatus. All the estimations were performed on the material, as removed

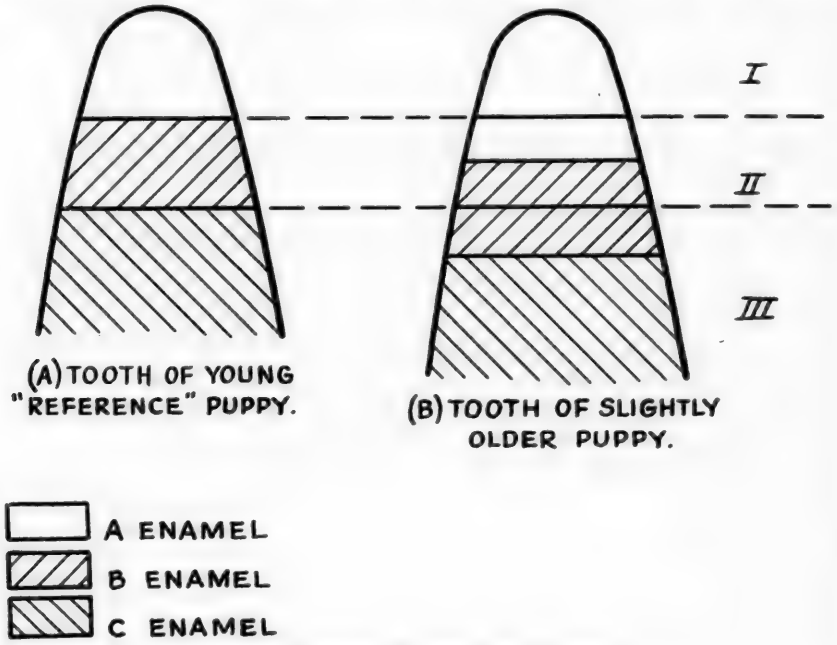


FIG. 3. Division of enamel into 3 regions

from the fresh teeth, without preliminary drying. The results are all, therefore, percentage values of the wet weight of the enamel. It should be mentioned here that although we aimed at keeping the enamel moist after its removal, pending weighing and chemical analysis, it cannot definitely be assumed that this enamel was in exactly the same state, as regards its water content, as the enamel *in situ*. The conditions make it possible to compare results from animals used in one litter more closely than those obtained from animals in different litters. In addition to the analysis of the teeth, the absorption of vitamin A was checked for each animal by the estimation of this vitamin in the liver.

[8] Carmine was used because it had been employed as a routine stain in previous work.

The serum calcium, phosphorus and phosphatase (King and Armstrong, 1934) were also determined, but no significant differences were observed. These results are not discussed in this paper.

Radiographic Method. A new, quantitative, radiographic method has been used, by which it is possible to determine the degree of calcification in terms of the actual percentage by volume of mineral matter present. The method is based on the determination of the linear x-ray absorption coefficient of the enamel by the photomicrometric examination of radiographs of tooth sections, and has been fully described elsewhere (Thewlis, 1940). It is important to realise that this method and chemical methods do not measure the same thing. Enamel must at first consist of protein matrix which can perhaps be regarded as a gel-like structure, the continuous phase being protein. This would enclose liquid within the meshwork. Calcification can then be regarded as proceeding by deposition of the mineral matter in this protein and would be accompanied by gradual encroachment on the liquid in the spaces. Chemical analysis measures the percentage mineral matter *by weight*. The radiographic method, on the other hand, measures the quantity of mineral matter *per unit volume*, and therefore takes into account to a greater extent than the chemical method the relative proportion of protein matrix to liquid in the spaces. According to Schmidt (1923/25, 1924, 1934), as calcification proceeds these spaces disappear, and it is therefore to be expected that the older the animals the greater will be the agreement between the chemical and x-ray results.

EXPERIMENTAL RESULTS

Litter 1. Control experiments to test the method of enamel separation. From the age of 4½ weeks 3 puppies of one litter were each given a good ordinary diet consisting of bread, meat and milk, with the addition for the last month of yeast and salt. The quantities eaten daily by each animal were as follows:

Bread	60–70 gm.
Scrap meat containing fat	40 gm.
Whole milk powder	25 gm.
Bakers' yeast	3 gm.
Salt	1 gm.

When 11½ weeks old the dogs were killed and the tooth-germs were taken from the right upper and lower jaws directly after death and as soon as possible the enamel was scraped from all the teeth in the 3 grades, A, B and C, described. In order to obtain sufficient material for the chemical analyses performed, for any given dog the enamel of each separate grade from the various teeth was pooled. Only a small amount of grade A enamel was present and that used was taken from the upper and lower carnassials, first premolars, canines and third incisors.

Carmine stained ground sections were made of the left upper and lower carnassials of dog 1, of the left lower carnassials of dog 3 and of every permanent tooth germ on the left side of both jaws of dog 2. The second stage of calcification was observed, however, in only some of the teeth in which grade A enamel had been found to occur. In this first experimental family, therefore, grade A

enamel apparently did not entirely correspond with the second stage of calcification. It must be remembered, however, that when there is very little stage II enamel it may be missed in ground sections as it is rarely possible to get a true median section.

The results of the chemical analysis of the enamel (all values given as a percentage of the wet weight of the enamel) are shown in Table I.

The percentage apatite was obtained by calculation from the calcium figure, assuming it to be all present as hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. It will be realised that the total percentage of inorganic matter is greater than the apatite figure given above, since there is present in the enamel approximately 1 per cent magnesium, traces of other metals and some carbon dioxide. The relative constancy of these chemical results for the 3 dogs indicated that the removal of enamel in three grades, A, B and C, could be adopted in future experiments.

TABLE I

Litter 1. Control experiments to test the method of enamel separation

DOG	AGE IN WKS. AT DEATH	EXPER. PERIOD IN WKS.	GRADE OF ENAMEL	PER CENT CALCIUM	PER CENT APATITE	PER CENT PHOSPHORUS	Ca/P	PER CENT MAGNESIUM
1	11½	7	A	35.50	89.1	15.24	2.33	1.15
			B	28.09	70.5	11.97	2.35	1.23
			C*					
2	11½	7	A	35.50	89.1	15.24	2.33	1.15
			B	27.20	68.3	12.64	2.15	1.23
			C	26.71	67.0	11.59	2.30	1.42
3	11½	7	A	35.82	90.1	16.09	2.23	1.10
			B	28.64	71.8	13.12	2.18	1.22
			C	27.76	69.8	12.52	2.22	1.38

* C enamel lost.

It will be noted that there is a great difference in each case between the calcium content of the A enamel and the rest. The small variations in the Ca/P ratios in the 3 grades of enamel are probably insignificant, but it seems that there is a gradual slight increase in the magnesium content from A to C enamel.

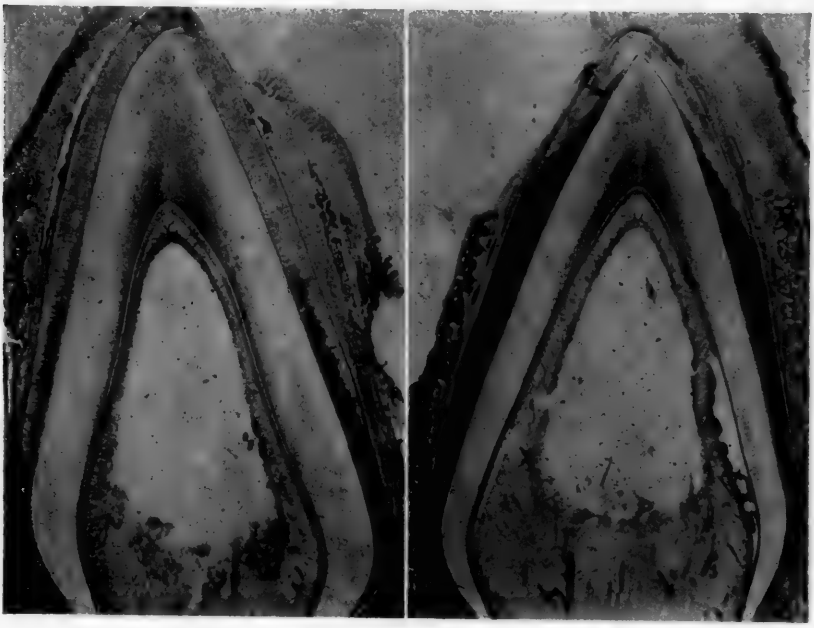
Litter 2. Comparison of the Effects of Cod liver Oil and Peanut Oil on Enamel Development. The object here was to study the teeth of dogs of different ages and to ascertain the effects of diet (cod liver oil as opposed to peanut oil) on them.

A litter of 6 puppies was used for this series of experiments. At the age of 7 weeks they were given the basal experimental diet which consisted of:

Bread	90–200 gm. per day
Meat	20 gm. per day
Separated milk powder	20 gm. per day
Yeast	3 gm. per day
Orange juice	6 cc. per day
Salt	1 gm. per day

In addition, 3 had 10 cc. cod liver oil and 3 had 10 cc. peanut oil daily. One pair of animals (1 cod liver oil, dog 4, and one peanut oil, dog 5) was killed at the age of 13 weeks, the second pair (dogs 6 and 7) at 17 weeks and the third (dogs 8 and 9) at 21 weeks.

Carmine stained ground sections were made of the left upper incisors and of the left upper and lower carnassials of each dog. At each age there was more stage II enamel in the teeth of the cod liver oil than in those of the corresponding peanut oil dog (*fig. 4*) and the stage of eruption was also further advanced. These differences were much more marked at 17 and 21 weeks than at 13 weeks, and



A B

FIG. 4. Photomicrographs (× 8) of carmine stained ground sections of left lower carnassials of the two 17 weeks old dogs of litter 2, dogs 6 (cod liver oil), A, and 7 (peanut oil), B, showing that in the dog receiving cod liver oil the degree of calcification of the enamel, as judged by the staining, is much more advanced.

there was more difference in the lower carnassials than in the upper carnassials and incisors.

Enamel was removed from the right upper carnassials for the estimation of calcium, phosphorus and magnesium. Material for the estimation of carbon dioxide was taken from several teeth.

In this family, dog 4, aged 13 weeks, which had received cod liver oil for 6 weeks, was used as the reference animal. Since, in these experiments we merely wished to obtain the maximum differences in the degree of calcification, only grade A enamel and enamel from the base of the tooth, consisting of grade C and the adjacent B enamel, were analysed in the reference animal. Similarly,

in the corresponding dog having peanut oil and in the pair aged 17 weeks, which had been on diet for 10 weeks, only regions I and III were analysed. In the oldest pair (aged 21 weeks and on diet 14 weeks) the enamel could not be scraped off and was not separated into different regions; the dentin was drilled out from the inside and the remaining shell of enamel analysed as a whole. This enamel consisted of grades A+, A and probably a trace of B. From Table II, in which the results of chemical analysis are given, it will be seen that both regions of enamel become increasingly calcified with age. At both 13 weeks and 17 weeks there is an appreciable difference between the calcium contents of the 2 regions. At 21 weeks, however, the whole of the enamel of the teeth of both dogs has probably nearly reached the maximum degree of calcification possible on the

TABLE II

Litter 2. Comparison of the effects of cod liver oil and peanut oil on enamel development

DOG	AGE IN WKS. AT DEATH	EXPER. PERIOD IN WKS.	ADDITIONS TO DIET	REGION OF ENAMEL	PER-CENT-AGE CALCIUM	PER-CENT-AGE APATITE	PER-CENT-AGE PHOS-PHORUS	Ca/P	PER-CENT-AGE MAGNE-SIUM	PER-CENT-AGE CARBON DIOXIDE
4	13	6	Cod liver oil	A	35.30	88.6	14.36	2.460		2.75
				C	30.20	75.8	14.36	2.103		2.75
5	13	6	Peanut oil	I	32.36	81.2	14.44	2.241		2.78
				III	29.21	73.3	15.18	1.925		2.78
6	17	10	Cod liver oil	I	36.18	90.8	15.65	2.313		
				III	32.08	80.5	16.27	2.014		
7	17	10	Peanut oil	I	35.69	89.6	16.19	2.204		
				III	31.33	78.6	14.60	2.151		
8	21	14	Cod liver oil	Whole (not separated)	36.62	91.9	15.85	2.310	1.08	
9	21	14	Peanut oil	Whole (not separated)	35.30	88.6	15.30	2.308	0.74	

respective diets. It is obvious that at each age both regions of the enamel of the cod-liver oil dog are more highly calcified than those of the corresponding peanut oil dog.

For radiographic examination the right lower carnassials of the dogs of this family were used. Longitudinal sections about 0.215 mm. in thickness, taken through the anterior cusp, were examined. A series of readings was made on each tooth, a central strip of enamel parallel to the amelodentinal junction, of length 0.5 mm. and breadth 0.1 mm., being examined for each reading. The results of the examination are summarised graphically in *figs. 5*, *6* and *7*. The ordinates in these graphs represent the volume percentage of mineral matter and the abscissae represent the distances along the enamel (measured from the tip) of the midpoints of the strips examined. These distances are given as

percentages of the distance along the enamel from the tip to the base, so as to render results obtained from different teeth roughly comparable. The labial and lingual sides of the teeth are indicated.

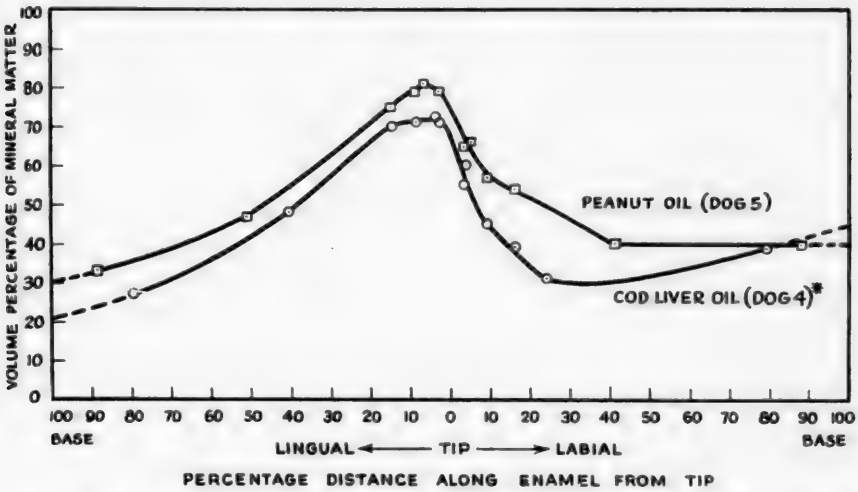


FIG. 5. The degree of calcification in the enamel of the 13 weeks old dogs after receiving the experimental diets for 6 weeks.

* This section did not pass exactly through the tip.

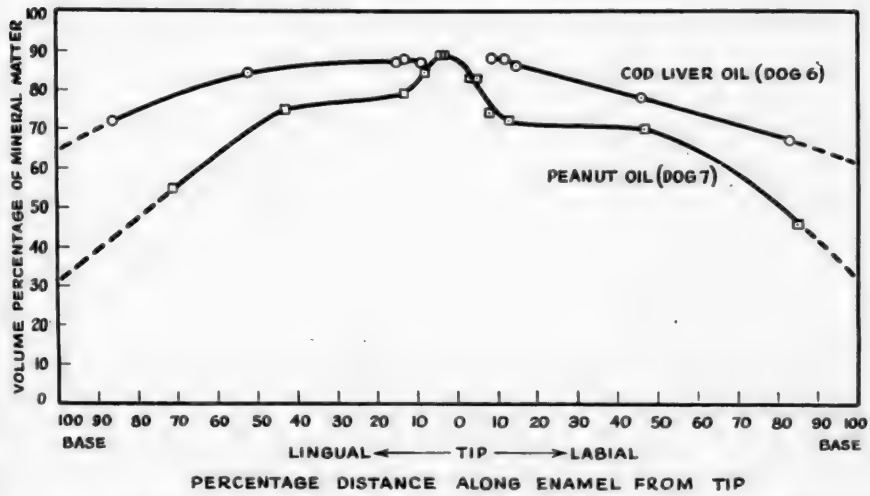


FIG. 6. The degree of calcification in the enamel of the 17 weeks old dogs after receiving the experimental diets for 10 weeks.

From *figs 5*, *6* and *7* it is possible to confirm that the tip of the tooth calcifies before the base. Visual examination of the radiographs confirms, too, that a strip of enamel along the amelodentinal junction is, in the early stages of develop-

ment more highly calcified than the bulk of the enamel. Further measurements (not shown in the curves) made at the amelodentinal junction indicate that this strip contains on the average about 10 per cent more mineral matter than the neighbouring enamel.

Before comparing the x-ray results for the cod liver oil and peanut oil dogs and correlating these with the chemical results, it must be remembered that, in contrast to the chemical results, which relate to all the enamel that can be collected from the different areas of the *upper* carnassials, the x-ray measurements give an indication of the state of calcification only in certain regions of one section (0.215 mm. thick) taken from a *lower* carnassial. It must also be borne in mind that in making sections, especially of teeth in the early stages of development, (as, for instance, at 13 weeks), it is impossible to be sure of getting exactly

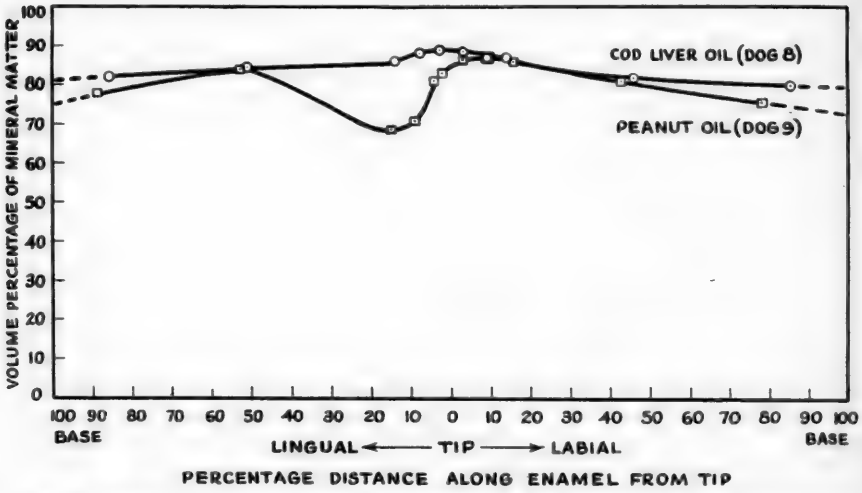


FIG. 7. The degree of calcification in the enamel of the 21 weeks old dogs after receiving the experimental diets for 14 weeks.

through the tips, where calcification is the furthest advanced. In later stages of development (i.e. at 17 and 21 weeks), the difficulty is not so great.

In the present series of measurements the chemical, x-ray and histological results show the same general tendencies for the older dogs, and the curves suggest that at 17 and 21 weeks (after 10 and 14 weeks of the experimental diet) the cod liver oil tooth is in each case more highly calcified than the peanut oil tooth. (The extreme tip of the tooth of dog 6 was broken off and no measurements could therefore be obtained from that portion.) For the 13 weeks old dogs (with a diet period of 6 weeks), however, the x-ray results suggest that the cod liver oil tooth is less highly calcified than the peanut oil tooth, whereas the chemical results (Table II) and the histological preparations suggest that the reverse is the case. For the reasons given above, too much stress should not be laid on the x-ray results. The apparent incongruity between chemical and x-ray

results is, however, capable of explanation when the structure of young enamel is taken into account. The two sets of results lead us, in fact, to the conclusion that the enamel of the cod liver oil dog is at an earlier stage of development than that of the peanut oil dog, but is, however, being built up of more highly calcified material. The greater calcium content of the "building" material of this enamel accounts for the chemical results, while its greater "dispersion" is the chief factor in the x-ray measurements.

Another feature at 17 and 21 weeks is the regular change in the degree of calcification along the tooth noticeable in the cod liver oil as compared with the peanut oil teeth. On each side of the tooth of dog 7 (peanut oil) (*fig. 6*) there is a region extending from near the tip to a point almost halfway along the tooth where the degree of calcification remains nearly constant; and in the tooth of dog 9 (peanut oil) (*fig. 7*) there appeared to have been a partial failure to calcify in approximately the same region, on the lingual side of the tooth. The x-ray, chemical and histological results obtained with this family confirm the conclusions reached in earlier experiments that cod liver oil promotes good calcification.

It will be noted from the above x-ray measurements that the quantity of mineral matter per unit volume of enamel is markedly less at the base than at the tip, particularly in the 13 weeks and 17 weeks old dogs. These variations are, in all probability, sufficient to account for the observed differences between the two regions. In other words, it is the quantity of mineral matter per unit volume which influences the hardness.

Litter 3. The Effect of Variations in the Vitamin A Content of the Diet in the Presence of Adequate Vitamin D. A litter of 4 puppies was put on to the following basal experimental diet at the age of 7½ weeks:

Bread	100–110 gm. per day
Meat	20–30 gm. per day
Separated milk powder	20 gm. per day
Olive oil	9 cc. per day
Yeast	3 gm. per day
Orange juice	6 cc. per day
Salt	1 gm. per day

This diet was supplemented by 2,500 I.U. daily of vitamin D (irradiated ergosterol) and 2 of the dogs received in addition large amounts of vitamin A (30,000 I. U. per day). One pair of dogs was killed at the age of 13½ weeks and the other at 16 weeks, after receiving the experimental diets for 6 and 8½ weeks respectively.

The teeth examined were removed from the right side of both jaws. The reference animal used for each 16 weeks old dog was that 13½ weeks old dog on the same diet. For chemical analysis regions II and III in the 16 weeks old dogs were subdivided further into grades. In region I a small amount of very hard enamel at the tip could not be removed by the method employed and was not analysed. In Table III, which indicates how the enamel was divided for chemical analysis, and in other tables, this is referred to as A+ enamel.

Examination of carmine stained ground sections of the left lower carnassials of these dogs showed that the second stage of calcification was present in each case but at both ages occupied a smaller area in the dog receiving vitamin A concentrate. This difference was most marked in the 16 weeks old pair of dogs.

The chemical results for this family are given in Table IV. It is seen that at both ages the enamel of the dogs receiving vitamin A was less highly calcified

TABLE III

AGE	NATURE OF ENAMEL IN		
	Region I	Region II	Region III
13½ weeks	A	B	C
16 weeks	A+* and A	A and B	B and C

* Not analysed.

TABLE IV

Litter 3. The effect of variations in the vitamin A content of the diet in the presence of adequate vitamin D

DOG	AGE IN WKS. AT DEATH	EXPER. PERIOD IN WKS.	ADDITIONS TO DIET	REGION OF ENAMEL	PERCENTAGE CALCIUM	PERCENTAGE APATITE	PERCENTAGE PHOSPHORUS	Ca/P	PERCENTAGE MAGNESIUM
10	13½	6	Vitamin D	A	35.26	89.59	15.35	2.296	1.31
				B	30.74	78.10	13.79	2.230	1.28
				C	28.24	71.73	12.47	2.265	1.93
11	13½	6	Vitamins A and D	A	32.48	82.50	14.59	2.225	1.64
				B	28.65	72.80	13.70	2.092	1.62
				C	27.00	68.60	13.12	2.060	1.78
12	16	8½	Vitamin D	I A*	34.62	87.96	16.13	2.144	1.43
				II A	32.48	82.50	14.80	2.194	1.70
				II B	28.57	72.60	12.97	2.203	1.19
				III B	27.38	69.56	12.54	2.183	1.21
				III C	23.55	59.83	11.30	2.084	1.38
13	16	8½	Vitamins A and D	I A*	33.64	85.43	15.44	2.178	1.53
				II A	30.02	76.25	13.78	2.178	1.22
				II B	27.61	70.15	12.28	2.249	1.37
				III B	27.24	69.20	11.94	2.282	1.43
				III C	25.65	65.16	11.61	2.210	1.07

* A+ enamel (see Table III) not analysed.

than that of the corresponding animals not receiving it. As in previous experiments the calcium figures for the enamel of these young dogs decreased steadily from the tip to the base of the teeth.

Litter 4. Further Experiments on the Effects of Additional Calcifying Factors in the Diet. In these experiments, which may be regarded as an extension of those made with litter III, either cod liver oil or peanut oil was included in the diet

and either vitamins A and D_2 or calcium carbonate were added to the diet as indicated in Table V.

At the age of 6½ weeks a litter of 6 puppies was given the following basal experimental diet supplemented by 6 cc. orange juice daily:

White bread	70 per cent
Meat	10 per cent
Separated milk powder	10 per cent
Yeast	3.5 per cent
Salt	0.5 per cent
Oil	6 per cent

TABLE V

Litter 4. Further experiments on the effects of additional calcifying factors in the diet

DOG	OIL IN DIET	ADDITIONS TO DIET	REGION OF ENAMEL	PERCENTAGE CALCIUM	PERCENTAGE APATITE	PERCENTAGE PHOSPHORUS	Ca/P	PERCENTAGE MAGNESIUM
14	Cod liver oil	None	A*	36.67	92.0	16.33	2.241	0.58
			B and C	30.19	75.8	13.30	2.270	0.61
15	Peanut oil	Vitamins A (15,000 I.U.) and D (2,500 I.U.)	I	33.88	85.0	16.05	2.111	0.78
			II and III	28.31	71.1	12.98	2.181	0.65
16	Peanut oil	Vitamin A (15,000 I.U.) (Some D in concentrate)	I	36.07	90.5	16.65	2.166	1.05
			II and III	30.83	77.4	13.80	2.234	0.81
17	Peanut oil	None	I	33.56	84.2	16.95	2.222	0.75
			II and III	29.87	75.0	14.05	2.209	0.63
18	Peanut oil	Vitamin D (2,500 I.U.)	I	36.93	92.7	16.67	2.216	0.79
			II and III	30.15	75.7	14.44	2.152	
19	Peanut oil	$CaCO_3$ (1% of total diet weight)	I	36.48	91.6	16.25	2.245	0.16
			II and III	31.07	78.0	14.33	2.200	0.57

* A+ enamel not analysed.

The total amount of food eaten was between 100 and 180 gms. daily and the proportions of the different ingredients remained constant. To dog 14 the oil was given in the form of cod liver oil (6 cc. being roughly equivalent to 3,600 International Units of vitamin A and 780 of vitamin D); to all the others it was given in the form of peanut oil.

The experimental period of 8 weeks was the same for all and the experiments were terminated when the animals were 14½ weeks old. No marked differences were seen in ground sections of the teeth.

In these experiments the enamel was analysed in 2 parts only, A enamel (or region I) and B and C enamel (or regions II and III) combined. The cod liver oil dog (14) was used as the reference animal. The chemical results for grade

A enamel in this dog are lower than would be expected, since some of the enamel at the tips of the teeth (termed A+ enamel) was too hard to be removed by the method of separation employed.

The results of the chemical analysis of the enamel of all the dogs of this family are given in Table V. The chemical results indicated that in relation to age the degree of calcification was high in the enamel of all the dogs except 15 and 17. One of these (dog 17) had received no vitamin or mineral addition and the other (dog 15) had received relatively large amounts of vitamin A and D concentrates. Lindquist (1938) states that the minimal daily requirements of man are 1,500 International Units of vitamin A; it would thus appear that 15,000 International Units per day for a dog might be excessive. This amount did not, however, seem to be detrimental when given together with a relatively small amount of vitamin D (see dog 16), but only when the diet contained a fairly large amount of vitamin D. These results, in addition to being compared with those for dog 14, may also be compared with those obtained in litter 2, where the vitamin additions, if any, were given in the form of cod liver oil. In this case, the degree of calcifition of the enamel was undoubtedly higher in the dogs receiving cod liver oil than in those with no vitamin supplements. It must be borne in mind, however, that in this series natural sources of the vitamins were given and the doses were considerably small than those given in litters III and IV.

The form of vitamin D produced by irradiation of ergosterol and called vitamin D_2 is not identical with that present in fish liver oils, which in all probability is chiefly the activated product of 7 dehydrocholesterol, called vitamin D_3 (Waddell, 1934, and Windaus, Lettre and Schenck, 1935). Biologically the 2 forms of vitamin D are known to have very different effects in rats and chicks (Massengale and Nussmeier, 1930, and Bills, Massengale, Imboden and Hall, 1937). These known differences might in part be the cause of the different results obtained in our experiments from the use of cod liver oil and vitamin concentrates.

SUMMARY

With a view to investigating the different stages in enamel development and the chemical and physical basis for the differences between good and defective (M-hypoplastic) enamel produced by different diets, the enamel of the developing teeth of dogs has been examined histologically, by chemical analysis and by x-rays. Prior to the present work, no investigation on developing enamel has been undertaken on the quantitative side either by chemical or x-ray analysis.

The investigation is a continuation of the work of Mellanby (1929), who differentiated, from the staining reactions of ground sections of developing teeth, 2 stages in enamel calcification, enamel in the "first stage" being soft and staining deeply and in the "second stage" being relatively hard and not readily stainable with carmine.

For the purpose of chemical analysis, enamel was divided into 3 grades (A, B and C), which were distinguished by differences in surface appearance and consistency. Grade A enamel was approximately at the second stage of calcification and B and C at the first.

The results of quantitative chemical and x-ray analysis indicate that the great differences in hardness observed in these grades can be attributed to differences in the concentration of mineral matter. The results also give quantitative support to the previously accepted view that, in the second stage of development, calcification proceeds from the tip downwards. Radiographic analysis also confirmed the finding that in the early stages of calcification a thin strip of enamel along the amelodentinal junction is more highly calcified than the rest.

The chemical results show that when the cod liver oil was included in the diet the calcification of the enamel was more advanced than in control animals receiving peanut oil, which is deficient in vitamins A and D.

In contrast to the beneficial effect of cod liver oil as a source of vitamins A and D_3, addition of vitamins A and D_2 together in relatively large amounts (2,500 I.U. vitamin D_2 and 15,000 and 30,000 vitamin A, including a little D_3) did not produce a greater degree of calcification than was found in the controls. When, however, these high doses of the vitamins were given separately, an increase in the degree of calcification occurred. It is felt that any deductions from the results which concern higher doses of vitamin A must be regarded as tentative pending further experiment.

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TOOTH ERUPTION: THE AXIAL MOVEMENT OF CONTINUOUSLY GROWING TEETH

HENRY SICHER

Department of Anatomy, Chicago Medical School, Chicago, Illinois

Many theories of tooth eruption have been propounded, modified, discarded and advanced again. The questions involved have not been answered satisfactorily (9). One of the reasons for this failure is the misconception of the term "force", pushing or even pulling the tooth out of its socket. That a very real force was meant is shown by the frequent reference to plant life. That a plant root can crack rocks, that a bean sprout can pierce the soil (and in experiments even thick paper or layers of tinfoil) have been compared to the cutting of tooth through bone and gingiva. This comparison is, however, absolutely false. The growing plant exerts pressure against its non vital surroundings. The erupting teeth, growing and moving in the environment of living tissue, are but parts of the organism.

This gross mechanical explanation for tooth eruption would isolate this process from all other developmental and growth phenomena. The "movements" of other organs in the body of the embryo are explained as results of differential growth. *Apriori* an explanation of tooth eruption in a similar way should be more satisfactory.

When speaking of pressure or pull exerted by a growing organ and consecutive changes in its relations to surrounding organs, these forces should be considered as minute differences in tension, as disturbances in the equilibrium just strong enough to act as stimuli for re-establishment of this equilibrium. The terms "bio-mechanical" or "bio-physical" stimuli could be introduced for these "forces" and the picture of gross mechanical changes eliminated.

A second reason for the insufficiency or the failure of hypotheses trying to explain tooth eruption is the frequent lack of a clear definition of the term eruption itself. Often only one or at least not all the movements of a tooth were considered. It must be clear, however, that all the different movements in exact correlation are necessary to bring a tooth into its normal functional position and to keep it in normal relations throughout life. These movements can be classified as follows: a, progressive movement in the direction of its long axis, *axial movement;* b, bodily movement of a tooth parallel to itself, *drift;* c, rotation of a tooth around any one of its transverse axes, *tilting;* d, rotation of a tooth around its long axis, *rotation.*

The anatomical structure of the tooth in different species or in different stages of its own development has to be considered too. The most important differences are found between teeth with limited growth and the continuously growing teeth, and in the former before and after full development of their roots.

A theory of eruption should, therefore, be formulated only after careful study of all the different movements of the erupting tooth and after examination of the behaviour of teeth of different anatomical structure.

This complexity of the problem can, perhaps, be overcome by breaking it down into separate questions. Therefore, the discussion in this paper will be limited to one movement of a certain type of teeth: the axial movement of continuously growing (and erupting) teeth during maturity. The rat incisor is an almost ideal object for such a study because its axial movement is so prominent and so fast that it can be studied and has been extensively studied grossly and histologically under normal and experimental conditions. A great number of facts on the biology of this particular tooth are available. Other continuously growing teeth were studied, e.g., those of guinea pig and rabbit.

CRITICAL REVIEW OF THEORIES

If all the theories on tooth eruption are considered, 3 factors remain which can be considered seriously as causing tooth eruption. They are: 1, Apposition and resorption of bone; apposition behind, resorption in front of the moving tooth. 2, "Vascularity". It is often not clearly expressed whether the mere presence of blood vessels in the alveolus can push the tooth out of its socket or whether a proliferation of vessels is seen as moving "force". 3, Root elongation. Here again, not much is said on details of this process, but it is mostly assumed that the apposition of dentin is the responsible factor. If these theories are examined in their value for the problem in hand, i.e., the axial movement of a continuously growing tooth, the first one can be eliminated immediately. The basal end of such teeth remains stationary in mature animals. This can be easily demonstrated by the constant relation of the basal end of the incisor to the molars, which offer a very good and convenient mark. Furthermore, no appositional growth of the bone at the alveolar fundus can be observed in histological specimens.

The second possible factor "vascularity" cannot play any role here either. First, in microscopic sections it can be observed that the periapical tissue is less rich in blood vessels than the other periodontal membrane on both the convex and concave surfaces of the tooth and that no proliferation of this periapical tissue or its blood vessels takes place. The "vascular" theory (1, 2) uses as a physical basis the fact that the root of the tooth is surrounded by vessels under a certain pressure and that the crown emerges into the oral cavity and is, therefore, under no tissue pressure. In this case the only force which would and could check the eruption of a tooth is the contact with its antagonist. If this were true, we could never find teeth without antagonists, e.g., the lower anterior teeth of ruminants; or teeth which never come into contact with their opposing teeth as the side teeth of most carnivores; or teeth which at rest are not in contact with each other as the incisors of rodents.

There remains by exclusion one factor only as cause for eruption: growth of the tooth in length. In other words this would mean that the growth of the tooth manifests itself as eruption, as far as it is not taken up by the actual growth of the tooth correlated to the growth of the animal itself. To see whether this

factor acts and in which way, it is necessary to undertake a detailed study of the growing end of such a continuously growing tooth.

Schour (5, 6), as proof against the theory that root elongation is the primary factor in the axial movement of the rat incisor showed, in hypophysectomized animals, that eruption slowed down and eventually stopped. Dentin production and therefore appositional growth of the tooth in length continued to a certain degree. The dentin was laid down in heavy folds which indicated some crowding in the region of the fundus. Schour's (6) explanation reads as follows:

> "In the incisor of the normal rat, dentin grows by apposition at the pulpal surface in form of layers which move anteriorly along the long axis of the tooth and are constantly replaced by similar layers. The balance between the rate of apposition and the rate of forward movement of these layers is such that in the mature rat, the total amount of dentin substance (in a given tooth) remains the same."
>
> "In the incisor of the hypophysectomized rat this balance is disturbed because of the more marked retardation of the rate of eruption. The result is that more dentin is being formed than is worn down, so that the dentin substance does not remain constant, but increases in amount. The new dentin layers that are being constantly and successively apposed cannot immediately occupy the position of the already formed and more slowly erupting dentin layers; thus in parts they accumulate at the expense of the pulp. Because of the more limited space at the posterior end of the tooth the dentin is here folded irregularly."

Further on: "This may indicate that the rate of tooth growth is less severely disturbed than the rate of eruption."

Schour, therefore, states rightly that dentin apposition cannot be regarded as the cause of eruption. But he wrongly identified dentin apposition with tooth elongation. I will try to show that tooth elongation is the cause of eruption and that Schour's classical findings have to be and can be re-evaluated.

OBSERVATIONS

At the proximal end of a rat incisor (*fig. 1*)—the so-called odontogenic zone—there is one peculiarity, the well known infolding of the enamel epithelia, Hertwig's epithelial sheath (H.E.S.) along the whole circumference of the tooth. H.E.S. forms in this way an iris shaped diaphragm narrowing the entrance into the pulp cavity to only half its width. The plane of this diaphragm is at an angle to the long axis of the tooth in such a way that the convex wall is much longer than the concave. This accounts for the fact that the angle of bending is smaller than a right angle on the convexity, very obtuse on the concavity. The inner layer of the diaphragmatic part of H.E.S. is formed by much higher cells on the convex side due to the development of enamel on this surface only. The buccal part of the diaphragm is shorter than the lingual so that the opening is eccentrically located and closer to the buccal side. This opening could, for convenience, be called the "epithelial apical foramen" (E.A.F.).

Closer scrutiny of the epithelial structures in the odontogenic zone shows that the cells of the inner enamel epithelium retain a considerable height almost to the line of reflection into the outer enamel epithelium. The decrease in size is gradual. The outer enamel epithelium is well defined in the diaphragm as a

single row of small cuboidal cells, which gradually lose their dense arrangement close to the bend and form a stream of 2 to 5 cell layers. In this arrangement they can be followed around the bend for a short distance until the ingrowing capillaries break up the outer enamel epithelium in an irregular folded layer. The space between inner and outer enamel epithelium is filled with several rows of small cells with elongated nuclei for half of the width of the diaphragm. These cells at first densely arranged become irregular and scarcer until well around the bend their inner layer forms the stratum intermedium.

On the diaphragm at the concavity the cells of the inner enamel epithelium are very short, at the best cuboidal, so that the transition into the outer enamel epithelium is less marked than at the convexity, otherwise the relations of the different layers is approximately the same. In the whole zone around the bend-

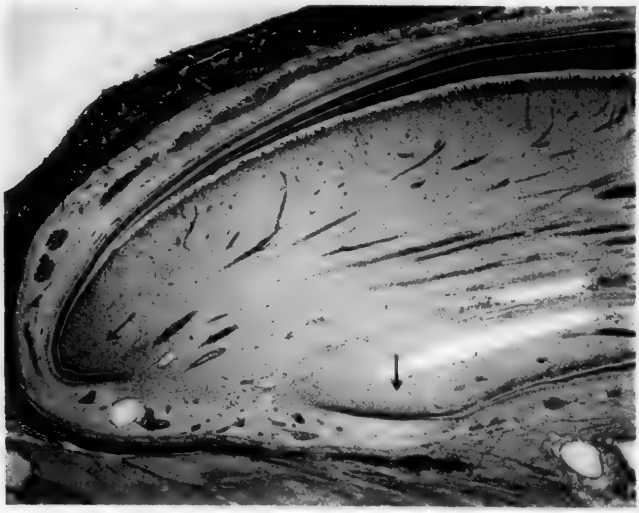

FIG. 1. Central longitudinal section through the basal end of upper incisor of white rat Arrows point to proliferation zones of pulp. Hemalum eosin stain.

ing numerous mitoses are found, always more in the region of the convexity of the tooth than in the opposite region.

The dentin ends on the convexity at quite a distance from the bend, but just reaches the bend on the concave side. The predentin on the buccal side ends still distal of the bending line and reaches on the concavity for a short distance into the diaphragmatic part and is therefore slightly bent itself.

The layer of odontoblasts, or of preodontoblasts, can be traced just around the bend on the convexity, the cells gradually losing height, on the concavity just past the apical end of the predentin in the diaphragm.

The pulp in this particular region shows 2 areas in which it is very rich in cells with well stained round nuclei. These cells are densely packed; capillaries are abundant. These zones contain numerous mitotic figures and are at once recog-

nizable as areas of proliferation. Silver impregnation shows here a much finer reticulum than in the well differentiated part of the pulp. The reticulum consists of fine precollagenous fibers. These zones have a different shape, relation and

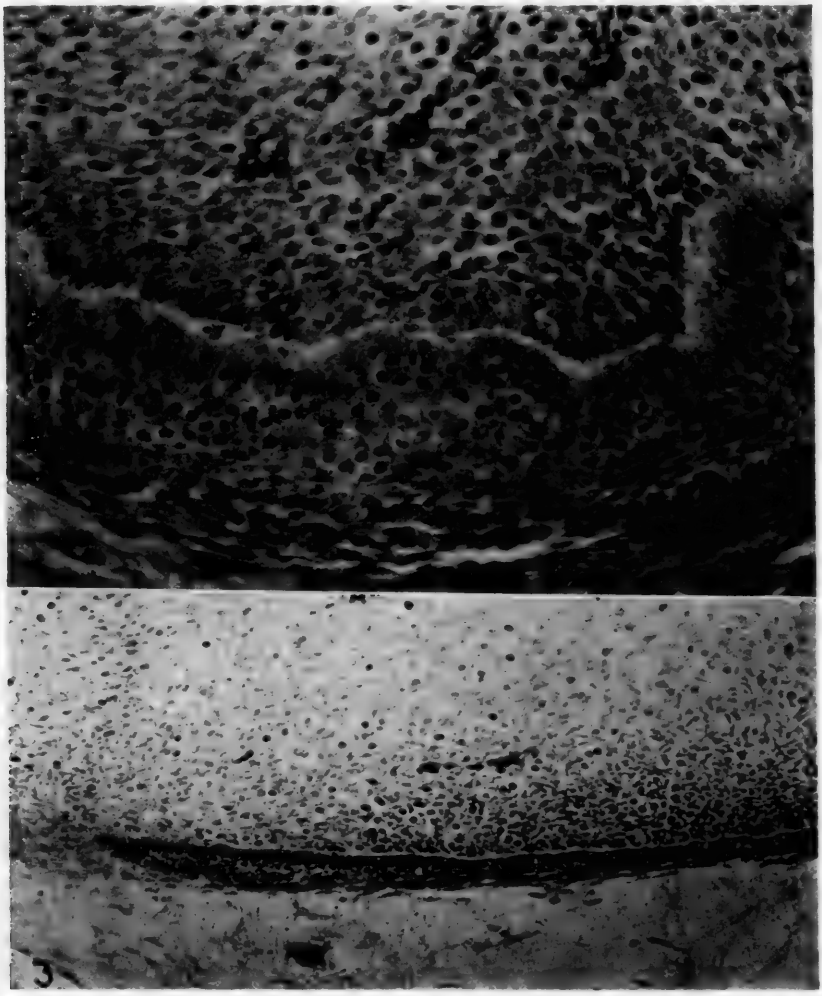

FIG. 2. Part of proliferation zone on convexity. Colchicin treated white rat. Numerous mitotic figures in epithelium and pulp. Iron-hematoxylin stain.

FIG. 3. Proliferation zone on concavity. Colchicin-treated white rat. Numerous mitotic figures in epithelium and pulp. Iron hematoxylin stain.

width on the opposite borders of the tooth. The area of proliferation on the convexity fills the whole corner between the straight and the diaphragmatic part of the enamel organ, on the concavity it is restricted to a rather narrow field just overlaying the diaphragm itself.

It is clear that there is only one zone of proliferation forming a ring around the E.A.F., and that the description of "two zones" applies only to the picture seen on a central section through a tooth. Therefore, this zone of growth is best termed the proliferation ring of the pulp.

On rats injected with colchicin (0.1 mg. to 100 g. body weight) the proliferation zones show numerous mitoses of young fibroblasts. In the mature pulp tissue a mitosis can be found only occasionally. The epithelium around the bend and in the diaphragm is in active proliferation too. The mitoses are more numerous in the stratum intermedium and stellate reticulum than in the inner enamel epithelium (*figs. 2 and 3*).

At the E.A.F. the pulp is separated from the periapical tissue by a narrow zone of more dense connective tissue.

The periapical tissue shows a clear differentiation into 2 areas. The layer immediately adjacent to the epithelial diaphragm and "closing" the E.A.F. is a

FIG. 4. Central longitudinal section through basal end of upper incisor of white rat. Three arrows point to hammock-ligament. Gomori's silver impregnation.

strong hammock-like ligament (*fig. 4*) which on labial and lingual side emerges out of the fibers of the periodontal membrane. Many of these fibers can be traced into the bone. Thus the proximal end of the tooth seems to rest on this ligament. This ligament is, of course, the basal end of the dental sac. Between this fibrous layer (perforated by the blood vessels entering and leaving the pulp and periodontal membrane) and the bone, the periapical tissue consists of very loose connective tissue which contains many larger vessels. Most of these, however, are on their way into the pulp. The periapical tissue shows no signs of proliferation.

The alveolar bone at the fundus is extremely thin, separated in the upper jaw from the bone of the maxilla by a layer of glands which in some specimens even reach the concavity of the tooth. These glands are very probably glands of the nasal mucosa. Most of the bone is in a resting stage.

Incisors of the rabbit, molars of rabbit and guinea pig give a picture similar in all major details.

DISCUSSION

The interpretation of these findings is very simple. It is known that tooth growth is initiated by the growth of H.E.S. The cells of the inner enamel epithelium induce the fibroblasts of the adjacent connective tissue of the pulp to differentiate into odontoblasts and then to produce dentin; formation of enamel or cementum are rather secondary features.

The first question is: where does the H.E.S. grow and how. A glance at the odontogenic zone of the rat incisor proves that the growth does not and cannot occur at the free end of the sheath. This would close the E.A.F. and would not contribute to the increase of the tooth length. The epithelial diaphragm, and especially the circumference of the E.A.F., must be considered as stable in its position relative to the long axis of the tooth. If mitotic multiplication of epithelial cells takes place between this free margin and the point where the predentin ends, then the distance between bending line and end of predentin will increase. These facts were already pointed out by Orban (3, 4). There is, however, necessarily a simultaneous growth of the pulp in this region as observation shows.

The pulp separated from the periapical tissue by the epithelial diaphragm and part of the hammock ligament, grows as an organ in itself, not by incorporation of periapical connective tissue but by interstitial growth in a circumscript and well defined area, the proliferation ring. This localized growth of the pulp in length is of exactly the same rate as the growth of H.E.S. Only by this co-ordination can the normal smooth shape of H.E.S. and the tooth itself be brought about.

The proliferation of the pulp as an organ in itself in co-ordination with the growth of H.E.S. precedes the apposition of dentin and is the important and leading process in tooth elongation. It is, at the same time, the "force" of eruption of many authors. It is biologically more correct to say that its growth is seen as eruption, as far as it does not contribute to the actual growth of the tooth.

There is still one question to answer. Why does the tooth grow out of its socket? The answer is provided by the hammock-like ligament on which the proximal end of the tooth rests. The pressure exerted by the growing tissue of the pulp acts in all directions. Bone is very sensitive to any increase in pressure of the surrounding tissue. It could barely resist without being resorbed, but connective tissue resists much better, and the hammock-like ligament, previously described, is the structure capable of giving a fixed, resistant basis for the growing tooth, so that it can grow only in one direction. It is to be remembered that this force of pressure should be thought of as small pressure differences acting less in a gross mechanical than in a bio-mechanical way.

The difference in the extent of the proliferation ring of the pulp on labial (convex) and lingual (concave) side of the tooth, is exactly correlated to the difference in the rate of growth and eruption on these 2 surfaces.

The growth of the tooth expressed by the growth of the pulp is, however, not the axial eruption itself. The tooth is connected to, and suspended from, the

bone. It is irrelevant whether this fixation is found on the whole circumference of the tooth or only on one—the concave—surface, as in the rat incisor. This fixation has to change continuously as the tooth erupts and a given point of its body is opposed to ever different points of the bone. The anatomical basis for such a continuous rearrangement of the suspensory fibers is the division of these fibers into 3 different layers (Sicher, 7, 8). In teeth of limited growth (man) these fibers, embedded on one end in the alveolar bone, on the other in the cementum, run directly across the periodontal area: principal fibers of the periodontal membrane. In the case of continuously growing teeth (*fig. 5*) the fibers coming from the bone and those coming from the cementum meet in an

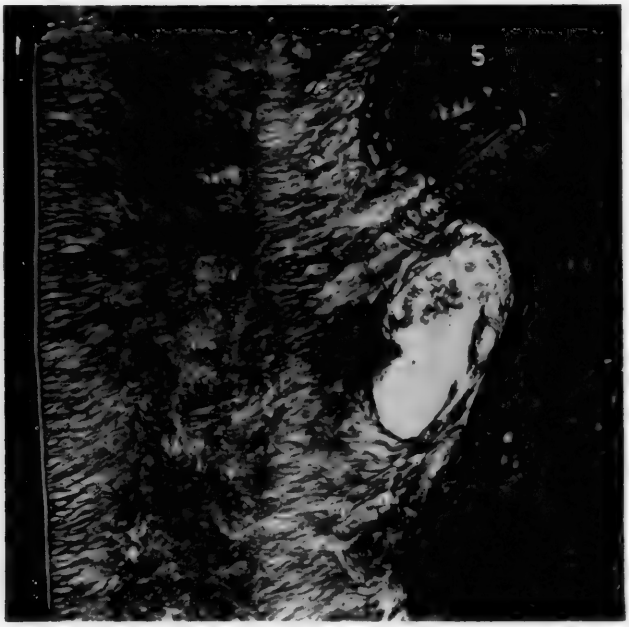

FIG. 5. Longitudinal section through lingual periodontal membrane of white rat incisor. 1. dentin (the adjacent white line represents the cementum); 2, dental fibers of the suspensory ligament; 3, intermediate plexus; 4, alveolar fibers; 5, alveolar bundle bone. Gomori's silver impregnation.

intermediate plexus. This plexus consists, as shown by silver stain, of precollagenous fibers, whereas the alveolar and the dental fibers are collagenous. Collagenous fibers can be traced into the intermediate plexus for a short distance. This arrangement allows a continuous rebuilding and rearrangement of the suspensory ligament without changes on bone and alveolar fibers or cementum and dental fibers.

This continuous change in the intermediate layer, the intimate details of which are still unknown, is an integral part of the axial movement of continuously growing teeth, and it might be said that the pressure differences resulting from the growing pulp act as bio-mechanical stimuli to enact just these changes.

An indirect proof of the validity of this explanation for the axial movement of continuously growing teeth is provided by the findings of Schour on hypophysectomized rats. The most striking facts he observed are: 1. Slowing down or stopping of eruption. 2. Folding of the dentin at the basal end of the tooth. If we look upon these facts in the light of our results, the simplest explanation is the following: the folding of the dentin has to be preceded by a folding of H.E.S. which plays the leading role in tooth formation both in a morphological and in a sense of sequence. The folding of H.E.S. is normally prevented by the co-ordinated pulpal growth. A break in this co-ordination in the sense that epithelial growth proceeds but pulpal growth stops can most easily explain the morphological findings of Schour. Such a difference in the growth of adjacent tissues causing folding of the faster growing parts can be found in numerous instances during normal development. It is one of the most important factors in organogenesis. The folded Hertwig's sheath still has the power to induce the differentiation of odontoblasts, and the pulpal tissue still has the power to respond to this stimulus. The newly formed dentin follows the folds of the H.E.S. and is therefore folded itself. The folding of the dentin and the inhibition of eruption of the rat incisor after hypophysectomy can both be explained most satisfactorily by the inhibition of pulpal growth in these animals. It is valuable and strong support for our conception of axial eruption of continuously growing teeth.

Our re-evaluation of Schour's findings on hypophysectomized rats, leads to another conclusion, which is already hinted at in Schour's (6) statement: "Hertwig's sheath displays greater vitality than any other dental tissue." We see the consequences of a dissociation of epithelial and pulpal growth. This means in other words: extirpation of the hypophysis inhibits mesodermal growth but does not inhibit, at least not to the same degree, epithelial growth. Whether this is a direct or indirect influence (via thyroid gland) remains to be seen. The importance of this different response of different tissues for a finer analysis of pituitary function is obvious. A second statement can be added, namely the observation that mesodermal tissue loses its proliferative capacity but not its power of differentiation. Further experiments will be necessary to get a clearer understanding of these relations.

It is well to point out that the conclusions drawn from these observations pertain only to the axial movement of continuously growing teeth. Whether teeth with limited growth show the same or a similar mechanism of axial movement during the formation and growth of their roots or whether other factors play the leading role will be discussed in a further study.

SUMMARY

The axial movement of a continuously growing tooth is the expression of its longitudinal growth. The leading factor in this orally directed growth is the elongation of the pulp. The pulp grows in a proliferation ring at its basal end. This proliferation zone is separated from the periapical tissue by an infolding of Hertwig's epithelial sheath, the epithelial diaphragm. The pulpal growth is normally simultaneous and equal to the elongation of Hertwig's sheath.

A "hammock" ligament on which the basal end of the tooth rests acts to direct the growth of the tooth. Continuous changes in the suspensory ligament of the tooth, stimulated by the expansion of the pulp, are an integral part of the process of eruption. These changes take place in the intermediate layer of the periodontal membrane. This layer consists of a plexus of precollagenous fibers.

Schour found in hypophysectomized rats inhibition of eruption and folding of newly formed dentin. Both these findings can be explained by a specific inhibition of pulpal (mesodermal) growth, whereas epithelial growth and the induction of dentin formation are far less affected.

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DENTAL CONDITIONS AMONG PREHISTORIC INDIANS OF NORTHERN ALABAMA[1]

SAMUEL RABKIN, D.D.S.

Cincinnati, Ohio

Although the primary purpose of these data is to cite collected evidence of dental conditions that existed among a prehistoric people, especially with regard to incidence of caries, pertinent information may be revealed concerning this much debated subject. As the ever-repeated discussions regarding the causes, incidence and the gloomy picture the teeth of modern man at present go on, this question comes to mind: what kept the teeth of prehistoric and primitive man from experiencing a similar fate? Admittedly this has a defiantly broad inference in need of qualifications. It is especially important since many authors deny that the teeth of early man ever were in a better state of health when compared with existing conditions. The latter challenging belief is definitely in need of qualification. To get these contradictory views parallel, it may be said that there is no difference of opinion or of facts based on contrasting evidence; primarily the source of information is to be considered. An experienced and well trained clinician will seldom make such an error. It is not uncommon for one not specializing in the dental field to erroneously place every damaged tooth in the category of caries. The fact that man's teeth during his known history were never caries free is well known and need not be discussed here. That the prevalence and incidence of caries between prehistoric, primitive and civilized society are not comparable is equally well known. No doubt the impression created by the morbid sight of dental disease (the teeth and jaws) of prehistoric man prompts some investigators to assume that this condition is the end result of caries.

True caries, to the limited extent that it prevailed in the distant past, was due to some deformity in the enamel, such as non-coalescent fissures, deep grooves, pits. In these food particles collected and ultimately resulted in damage to the tooth. The frequency of such occurrences evidently was not high as is revealed by most of the collected skeletal remains. There is less to be said in favor of the state of health of the jaws. This was a deplorable condition which no doubt caused early man no end of trouble. In contrast to caries, disease of the jaws was prevalent to a greater or lesser degree among ethnic groups at all times. While some dental anomalies, disharmonies and aberrations occurred frequently among man through the ages, somatic and structural characteristics, including those of the masticatory system, appear to have been always very diversified. Striking distinguishing structural variations occurred

[1] Received for publication November 13, 1941.

frequently among some aborigines in the same locality and period, but these variations became more apparent with the advance of civilization. Departures from the spaciously shaped mouth and large teeth to shortened jaws, either with continued large teeth (megaladontia) or with a proportionately smaller size of teeth prevailed among early and very primitive peoples. This is frequently found among the assembled remains of the Negroids, Australians, Tasmanians, peoples of various parts of Africa including Egypt, Eurasia and the American Indians. The same is true with regard to the gradual development of progressive dental pathosis of which malocclusion, next to caries, began to manifest itself differently among some people. Environmental factors, inadaptive circumstances, contact or direct effects of civilization have led to existing dental conditions today. In view of the prevailing perplexities concerning the causes of caries, or other entrenched dental disease, inquiry into the foregoing mentioned phases is essential. Such an introduction is especially important before an attempt is made to account for diverse existing conditions within a certain group.

Excluding the above, an account would restrict this investigation to technical terminology and the enumeration of figures. Also, this would limit availability of information to those engaged in the inquiry of the dental problem as a contrast between the past and the present. Moreover, such a restriction would eliminate vital information that may shed light upon the contrasting phenomena between the earliest and the succeeding groups of the same people as revealed by the skeletal remains.

Since the study of the dental problem through the history of man depends largely upon the evidence provided by such assembled material, every available opportunity to supplement needed information must not be overlooked. This is especially important since the various stores of collected remains regardless of origin, source, period or location of the people all disclose the same type of evidence, *i.e.*, existing contrasts between ideal and morbid conditions and diversity in the morphologic pathosis. It is curious that in the past, as in the present, the same conditions prevail whereby some individuals are immune while others suffer from dental disease. The Northern Alabama material would, upon first glance, give evidence of containing such a riddle. However, this material does provide ample basic evidence that accounts for existing diverse physical and dental conditions among similar groups of people separated by time and space.

During their early settling in this hemisphere the Indians undoubtedly had no contact with white man or his civilization, and an ample source of wholesome food supply may account for the low incidence of dental caries among Shell Mound folk in contrast to the successors of the same people during later periods. Chronologically, the collected remains of the Northern Alabama Indians represent the inhabitants of this area in continuous succession dating back from well over a thousand years ago for the Shell Mound folk to the Koger Islanders of a much later period, perhaps between 1500–1650 A.D. Corresponding observations may be found upon investigation of all other sources of similar material

revealing the fact that the earliest prehistoric peoples show the least incidence of caries. Extensive damage to the jaw bones and alveolar abscesses were experienced by most prehistoric peoples. These invariably resulted from excessive wear of the teeth caused by sand and grit in the food, culminating in exposure and death of pulps followed by disease of the bone. Even this process of tooth destruction, resulting from attrition, differs in many respects depending upon the rapidity of the wear of the teeth, the abrasive material that found its way

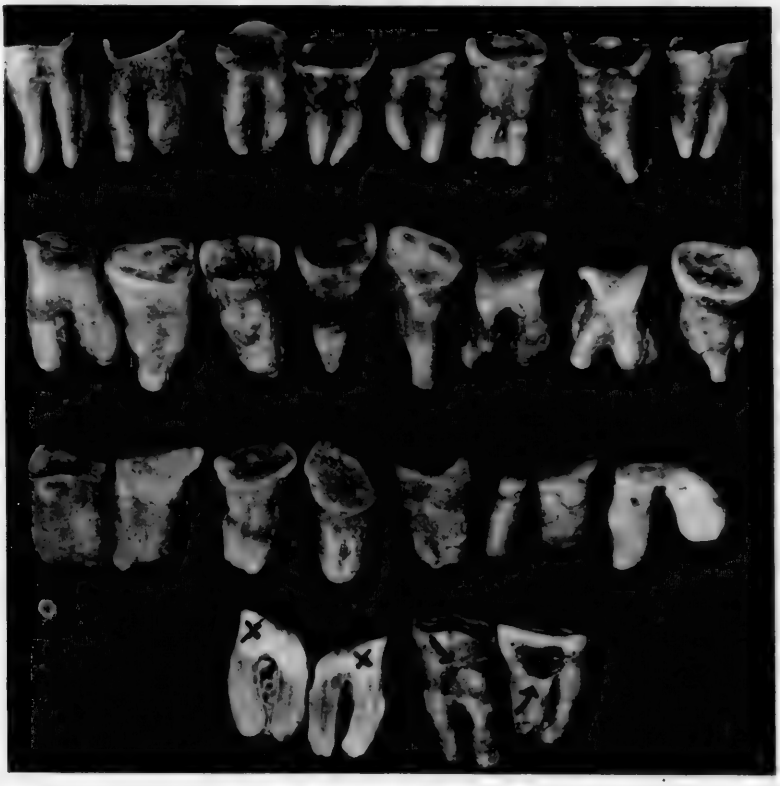


FIG. 1. Various stages of attrition due to coarse food. Worn surfaces are highly polished and, in most instances, pulp chambers and canals are solid masses of secondary dentin as revealed in sectioned specimens (×). Interproximal decay in 2 teeth adjacent to sections.

into the food, and other factors. Regardless of the degree of attrition suffered by the teeth, it is found that in many instances the wear is relatively uniform, indicating a slow process allowing secondary dentin to form within the receding pulp chamber and root canals (*fig. 1*). Alveolar abscesses in such instances are rather rare. The opposite occurred in other instances where rapid wear of the teeth began early in life resulting in premature loss of some of the teeth through disease of the pulps, or retention followed by extensive damage.

It is the excessive and rapid attrition which usually leads to loss of structure, uneven wear of the teeth and the morbid picture usually revealed by such material.

Incidental deformities and dental aberrations have occurred similarly in man and beast at all times. The same is true with regard to alveoclasia which is the end result in man and beast during the declining years of life. Therefore, when evidence is cited that early man had his share of dental troubles, it should be understood that it is not the tooth of the early human was inherently predisposed to decay, but that it suffered chiefly from mechanical and extraneous causes. Indeed, there have always been some isolated instances where actual caries did occur in the earliest known humans.[2] However, the teeth of prehistoric and primitive man were more resistant and the quality of the tooth made it less predisposed to decay. Evidence of a prevailing predisposition towards structural alterations and greater frequency of degenerative changes became more manifest among many ethnic groups with the advance of civilization. For that reason when analyzing the contrasting differences in the dental conditions between the earlier settlers and later populations of northern Alabama these factors are to be considered:

(1) That many physical and, no doubt, environmental changes had taken place during the intervening periods (between the earlier and later groups) all of which influence the structure, composition and predisposition of the teeth and jaw bone to degenerative changes.

(2) That, no doubt, intervening cultures and changes in food habits from pre-agriculture, consisting of marine and land animals, to a mixed diet of flesh and carbohydrates made the teeth more susceptible to caries. Especially is this true since it is generally believed that particles remaining around and between the teeth from carbohydrate food will be decomposed and fermented and will cause decalcification of the enamel. This phase becomes clearer when observing that the teeth of these Indian children were free from decay in contrast to adults in whom recession of the gum and alveolar process permitted food to collect and cause decalcification of the contacting teeth. Very little is known of the soil chemistry and the mineral elements (calcium and phosphorus) it provided for bone and tooth structure through the foods cultivated by the later groups.

(3) Whatever influence the factors mentioned may have had on tooth composition and predisposition to decay, a change in the dental morphology followed from the early to the last-known settlers. These consisted of transformations in the shape, size and pattern of the jaws and teeth to numerous variations gradually acquired throughout the intervening cultures.

As for the source and the type of food upon which the early settlers subsisted, reference is made here to appended information submitted by Webb and Snow.[3]

"(SILVID-SHELL MOUND. The excavation of extensive shell heaps reveals a preponderance of shell fish and fish remains supplemented with many varieties of turtles and great numbers of water fowl and small mammals. Ortmann in working out the faunal distribution of the fresh water mussel found that along the Tennesee there is a remarkable overlapping and union of at least 83 species, 29 genera of shell fish present in northern Alabama. It seems that the cold and warm water fauna (Cumberland and the Ohio) mixed

[2] Rabkin, S., Tooth Decay. *J. Medicine* **21**: 488, 1941.
[3] Personal communication.

here in astonishing quantities. And where there were shoals full of fish there were Indian remains. Thus the diet was heavily protein, based on the shell fish, fish, crayfish, turtle, then a dominance of bird bone remains indicate water fowl such as ducks, geese, swans, herons, etc., small mammals like the fox, raccoon, opossum, rabbits, groundhogs, squirrels (few deer, bear, lion) etc., and upon no evidence, it seems reasonable to assume that they gathered whatever in the line of fruits and nuts and vegetables the woods offered. The people are regarded as simple hunters and gatherers with no known form of habitation or houses.)

"(CENTRALID-CENTRAL KOGER ISLAND. These people, leading probably a semi- or possibly a sedentary life, based it upon agricultural staples. At any rate the diet was more varied and included meat from the larger mammals as deer, elk, bear and mountain lion along with the usual small mammal list given above. Wild turkey bones have been found in many prehistoric sites. Now this agricultural data rests upon some of the remains found at Moundville, and other sites, which present shell-tempered pottery shards. This same kind of pottery and designs were reported by the early historic explorers in association with the Choctaw, Chickasaw, Natches Indians who were sedentary agriculturalists. They had a more varied diet based largely upon agricultural products such as maize, beans, squash, berries and vegetables from the woods, supplemented by the meats of fish, shellfish and game.)"

In connection with the morphologic changes of the human teeth among an ethnic people, the following evidence is pertinent. It may be stated that the dentition is an exponent part of the body which reveals ontogenetic modifications as well as the tendency for the continuation of hereditary characteristics. Much depends on the predominating biologic inherent factors and extraneous environmental influences. Any cause for departure from the typical original characteristics will result in newly-acquired modifications especially if overwhelmingly adverse influences persist. Some of the original traits are at intervals singled out, and other characteristics are often lost. This can be observed in the distinct pattern of the large "shovel shaped" incisor with prominently raised marginal ridges of the enamel of the mesial and distal surfaces of the tooth, prominent cingulum and large primitive-patterned posterior teeth predominant in the Shell Mound folk. Other striking features are the flat contact mesial surfaces of the upper first incisors hardpressed against each other (*fig. 2A*). The second incisors are also very large and resemble the first incisors. Some evidence of these characteristics occasionally recurred among some of the later groups, but extensive structural modifications and variabilities became more wide spread in contrast to predominant constancy among the Shell Mound group. Not only do some of the teeth of the later groups resemble the shape and structure of those of modern man, but also, by comparison, the quality and composition of the enamel and dentin were found to vary. Aside from the fact that the teeth of the earlier group were larger and more primitive, the enamel has more resistance to cutting and the dentin seems denser than most of the specimens from those of later periods. Obviously, increases in population, shifting and territorial expansion, associated with other diverse concomitant influences culminated in an array of newer tooth patterns. While this process of modifications no doubt was slow and gradual, it is nevertheless difficult to ascertain the break in continuity from the predominating pattern or from the typical to variant. In the absence of any other evidence, it may be assumed that an abundance of

calcium and phosphorus in the food consumed by the earlier group may have been the predominating factor for the superior quality of their teeth. The few cavities present in earlier specimens were due to defects in the enamel and confined to "pit and fissure" grooves allowing food retention which, decomposing, resulted in tooth decay. This process of decay is definitely unlike the decalcified type of tooth predominating among modern man (*fig. 2B*). That environment and food derived from varied sources, including soil composition, have a definite

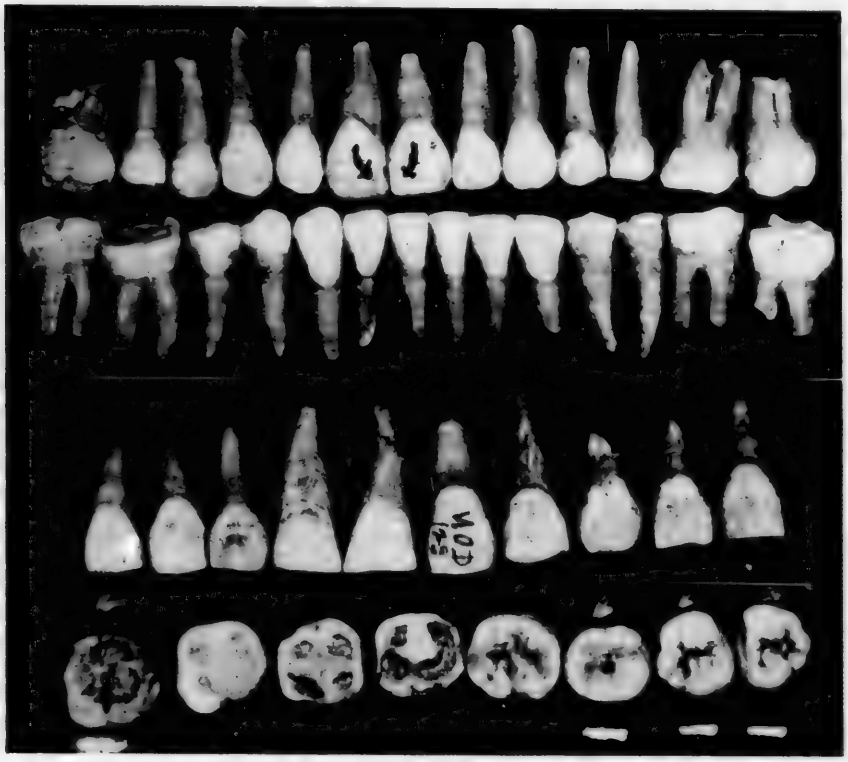


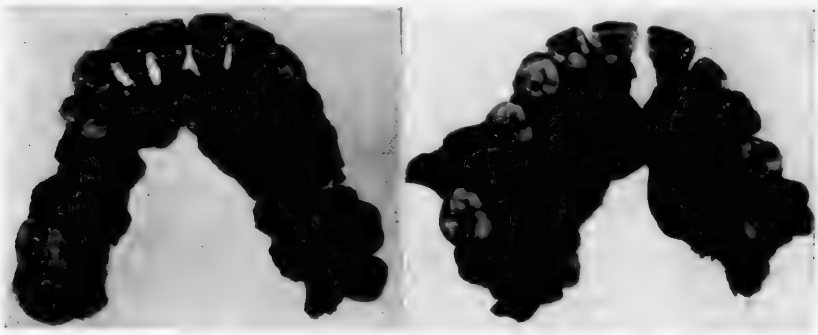
FIG. 2. A—Complete set of primitive teeth. B—Assorted specimens of "indeterminates." Defects in enamel leading to tooth decay indicated here by check marks (slightly reduced).

influence on bone and tooth structure and predisposition to diseases may be observed by comparing the above groups with some of the specimens of the Madisonville, O., and northern Kentucky Indians. These reveal conditions not unlike those found among modern civilized man.

Two striking illustrations cited here (*figs. 3* and *4*) similarly indicate transformations in structural constancy and predisposition to disease as a result of cumulative, diverse, and adverse influences. In both instances, the individuals belonged to the later inhabitants of Centralid types. The tooth structure and

susceptibility to caries definitely did not differ from conditions prevailing at present. This, however, is not to serve as a deduction that the human dentition of the past for the most part did not differ from the present. It is also not intended to give the impression that the later groups were the direct descendants of the earlier stock.

To the student of dental pathology, this account should provide consistent information regarding the obscure picture of the human dentition between the distant past and the immediate present. Regardless of prevailing circumstances,



A B

FIG. 3. A—Age 24–25, (K. I.—Lu° 25—430) Caries similar to that of modern man. B—Age 19–20, (N. S. M.—Lu° 59) Missing premolars and decay in left molar.

A B

FIG. 4. A—Age 15–17, female—retroverted first incisor. B—Radiographs disclosing interproximal decay affecting most of posterior teeth.

physical and physiologic modifications are the result of cumulative, inherent and environmental influences which generally include the known genetic forces governing blending of diverse types. The time element is not a factor and radical changes do not occur spontaneously. Tables I and II will give a more vivid picture of the transitory dental changes from very early man through succeeding generations depending upon numerous inherent and extraneous factors responsible for ensuing alterations.

Other evidence of occurring abnormalities and pathologic conditions besides

TABLE I

Existing dental conditions among Indians of Pickwick, Wheeler and Guntersville Basins

LOCALITY	SEX		TOTAL NO. OF INDIVIDUALS	PER CENT	INDIVIDUALS WITH:			
	Male	Female			Caries	Per cent	Alveolar Abscesses	Per cent
Silvids—Northern Alabama Shell Mounds								
Pickwick Basin	M.		298	40.5	1	0.34	34	11.41
" "		F.	253	34.4	3	1.19	45	17.79
Wheeler Basin	M.		65	8.8	3	4.62	10	15.38
" "		F.	56	7.6	2	3.57	12	21.43
Guntersville Basin	M.		30	4.1	2	6.67	6	20.00
" "		F.	34	4.6	3	8.82	4	11.76
Totals			736	100.0	14	1.90	111	15.08
Centralids—(Mississippi Culture) People—Koger Island Inhabitants								
Pickwick Basin	M.		169	28.5	10	5.92	16	9.47
" "		F.	111	18.7	15	13.51	20	18.02
Wheeler Basin	M.		53	8.9	0	0	5	9.43
" "		F.	46	7.8	1	2.17	2	4.35
Guntersville Basin	M.		110	18.6	5	4.55	22	20.00
" "		F.	104	17.5	8	7.69	12	11.54
Totals			593	100.0	39	6.58	77	12.98
Indeterminate physical types—Northern Alabama Shell Mound and Mississippi Pooled (Not the same series as in other tables)								
Pickwick Basin	M.		352	22.3	3	0.85	13	3.69
" "		F.	252	16.0	7	2.78	9	3.57
Wheeler Basin	M.		91	5.8	0	0	4	4.40
" "		F.	80	5.1	0	0	4	5.00
Guntersville Basin	M.		413	26.2	14	3.39	10	2.42
" "		F.	388	24.6	19	4.90	3	0.77
Totals			1,576	100.0	43	2.73	43	2.73

Total number of specimens available for examination—2905. Grouped they comprise the *Silvid* (Northern Alabama Shell Mound), *Centralids* (Koger Islanders), and *Indeterminates* (latter an assembled collection consisting of untypable, mixed specimens pooled from above groups without indication of distinct identity) both morphologically and archaeologically.

Group chronologically	*Silvids*—earliest group; estimated period circa 1000–2000 years (estimated and submitted by Snow and Webb[3]).
	Centralids—late prehistoric (Koger islanders); estimated period circa 1400–1600 (estimated and submitted by Webb and Snow[3]).
Culture	*Silvids*—pre-pottery, preagriculture, pre-bow and arrow, atlatl (throwing stick).
	Centralids—pottery, agricultural, sedentary and other cultural development (bow and arrow, etc.).
Food Habits	*Silvids*—(principally flesh), land animals, fowl and fresh water provisions (fruits?).
	Centralids—(combination cultivated produce) meats.
Territorial Locality Designated for Both Groups	Pickwick Basin Wheeler Basin Guntersville Basin

[3] Personal communication.

TABLE II

Existing dental conditions according to age of individuals

Incidence of caries and abscesses, indicating contrasts between earliest and later groups

	SILVIDS NORTHERN SHELL MOUND				CENTRALIDS ROGER ISLAND TYPE			
	Males		Females		Males		Females	
	Caries	Absc.	Caries	Absc.	Caries	Absc.	Caries	Absc.
	No. Ind.	No. Ind.	No. Ind.	No. Ind.	No. Ind.	No. Ind.	No. Ind.	No. Ind.
Pickwick Basin								
Child								
Adolescent							1	
Sub. Adult								
Young Adult	1	18	2	39	5	13	10	19
Midl. Adult		14	1	6	4	3	3	1
Old Adult		2						
Total	1	34	3	45	9	16	14	20
Wheeler Basin								
Child	1							
Adolescent								
Sub. Adult								
Young Adult	2	4	2	12		1	1	2
Midl. Adult		6				4		
Old Adult								
Total	3	10	2	12		5	1	2
Guntersville Basin								
Child						1		
Adolescent			1				2	
Sub. Adult							1	
Young Adult	2	2	2	3		8	3	9
Midl. Adult		3		1	5	12	1	2
Old Adult		1				1		1
Total	2	6	3	4	5	22	7	12

INDETERMINATES

	Pickwick Basin				Wheeler Basin			
Child	2	2						
Adolescent								
Sub. Adult								
Young Adult	1	11	4	5		4		1
Midl. Adult			3	4				1
Old Adult								
Total	3	13	7	9		4		2

INDETERMINATES

	Guntersville Basin							
Child	1		1					
Adolescent	1		2					
Sub. Adult								
Young Adult	6	5	12					
Midl. Adult	3	4	3	1				
Old Adult	1	1						
Total	12	10	18	1				

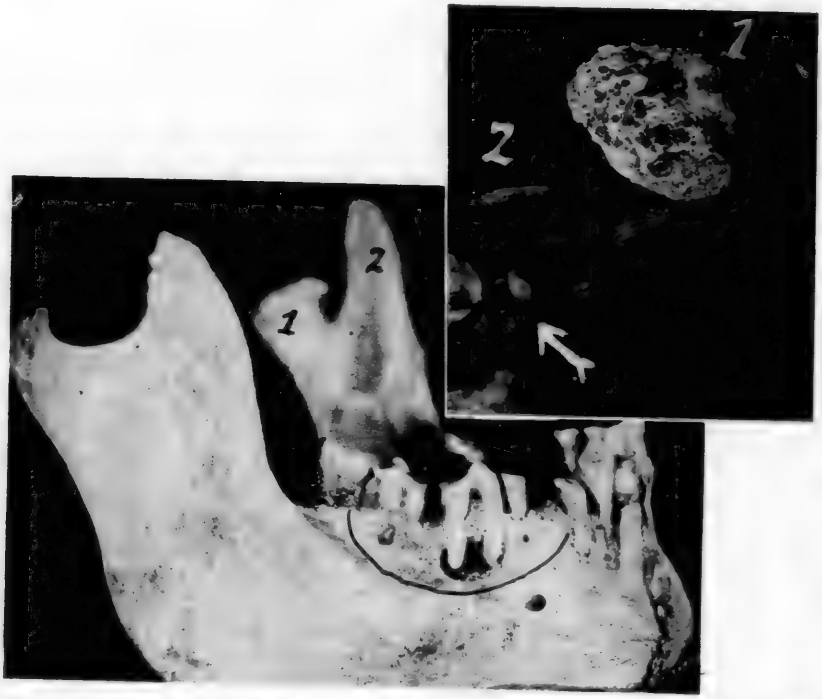


FIG. 5. Aside from the abscess cavity and generalized alveolar disease, this mandible reveals many other pathologic conditions. Osteoarthritis (inset enlarged) of left condyle (1) and deformed coronoid process (2); partially emerging third molar pressing against second (arrow).

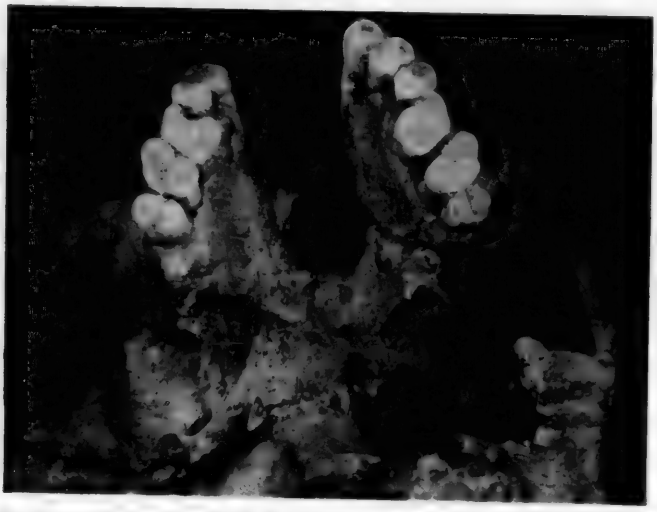


FIG. 6. Perforated palate and healed border of bone suggest possibility of a gumma; similar lesions are discernible in other parts of the skeletal frame among many specimens.

those listed consisted of impacted, malposed, rotated, and retroverted teeth. Abscess cavities in the jaw bone resulting from diseased pulps varied in shape, size, and depth within the bone, depending upon the duration of time the offending tooth remained in place. Many other types of bone disease occurred among these people. Evidence of osteoarthritis appears to have been common among them (*fig. 5*). Bone pathosis of indeterminate origin variously suggesting the possibility of syphilis, yaws or actinomycosis and involving different parts of

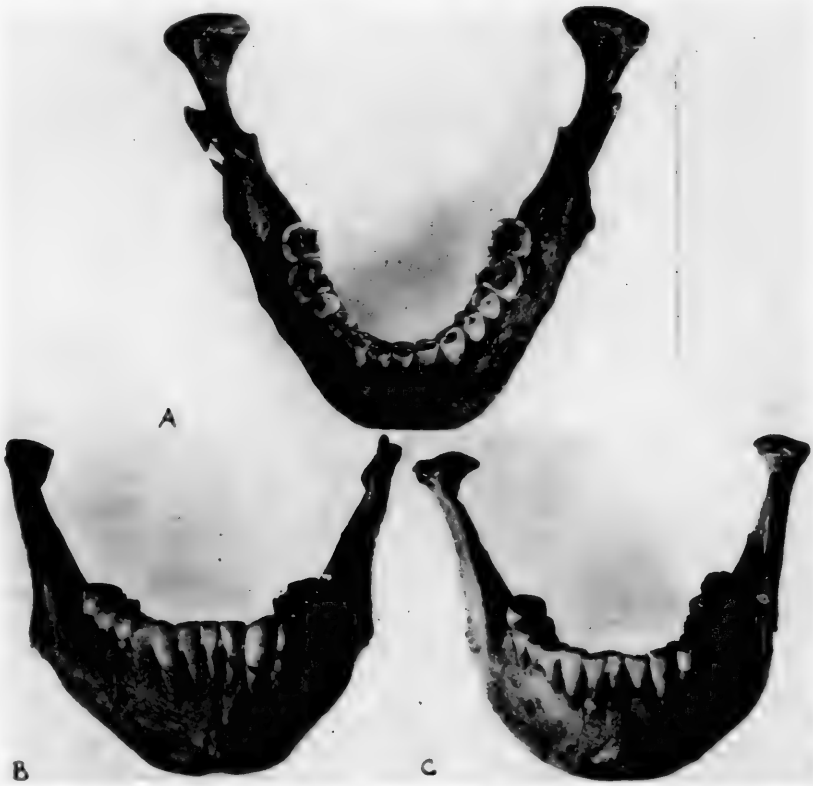


FIG. 7. A—Northern Shell Mound. Male. Age 21. This specimen generally represents ideal conditions. Attrition of teeth is not extensive, but uniform. In most instances teeth of earlier groups appear to be highly mineralized, most hard to cut and of a bluish white tinge. B and C—Northern Shell Mound. Female. Age 20. For corresponding chronologic age these specimens comparably lack the outstanding features revealed in A.

the body is found among the specimens. Some illustrated examples of such conditions involving the jaws are included among the list of illustrations.

SUMMARY AND CONCLUSION

Although the primary purpose of this account is to present pertinent evidence of existing dental conditions among a people of the past, an object lesson may be had if this investigation is applied to the inquiry into the causes of dental disease today. This will reveal that:

1. Not all observed damage to the jaw bone among specimens examined was the result of direct tooth decay. 2. Alveolar abscesses were the result of excessive and extensive attrition of the crowns of the teeth (from sand and grit in the food) leading to death of the pulp with subsequent disease of the bone; such attrition does not occur in modern man's teeth. 3. The incidence of actual tooth decay was relatively small among men of the past and definitely not comparable to conditions as they now exist. 4. The teeth of earlier man were of better quality and probably contained proper proportions of the essential chemical elements, some of which today may be disproportionate in the teeth of civilized man. 5. The term "caries" is too vague, if not inappropriate, for a distinct interpretation of the various types of tooth destruction, and cannot all be included in a specific pathologic category. 6. Acquired predisposition to tooth disease in modern man before eruption, and complicated (after formation) by a host of causes after eruption, should not be confused with incidental imperfections in the formation of the enamel. In the former the tooth breaks down because of lack of resistance, as though suffering a chemical change, in the latter it is affected by the collection of food particles within the recesses, leading to tooth decomposition and decay. That the saliva and excessive carbohydrate food consumption is invariably responsible for tooth destruction is revealed by cited evidence and is known to affect teeth today. It is to be observed that the onset of decay is usually on the surfaces where food is allowed to collect and that the decay in most instances does not continue after such teeth are crowned (gold or porcelain).

The question of proper food habits and nutrition cannot be over-emphasized, but since the prevailing predisposition to tooth degeneration is of cumulative origin, improved conditions cannot be instituted at will and in short order. Still less may be gained by feeding a child supplementary restoratives after the full formation of the crowns and eruption of the teeth. An adequate diet, that will provide the necessary mineral elements for sound teeth and bone, cannot be had unless all sources of food supply abundantly contain these vital elements. Moreover, our entire concept regarding the major causes of tooth decay will have to be subordinated to a biologic interpretation of the static and dynamic factors which have resulted in radical structural alterations, as well as persistent predisposition towards degenerative changes. Therefore not only the inherent properties of the first body cell must be considered, but also how it will be affected by later environmental influences.

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SENSITIZATION OF RABBITS BY INTRADERMAL INJECTION OF HEAT-KILLED LACTOBACILLI OF HUMAN ORAL ORIGIN[1]

NED B. WILLIAMS, D.D.S., AND R. W. HARRISON, PH.D.

Walter G. Zoller Memorial Dental Clinic and Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.

Examination of the reported findings of many investigators who have studied the incidence of lactobacilli in the oral cavity reveals that the cultures isolated have usually been regarded as belonging to a more or less homogeneous group, presumably a single species. These organisms have been referred to as *Bacillus necrodentalis* (1), *Bacillus acidophilus odontolyticus* (2), *Lactobacillus acidophilus* (3), and less specifically as oral lactobacilli (4a). While there have been, insofar as the authors are aware, no extensive investigations on the origin of the oral strains, it is usually assumed that they enter the mouth in food. Since more than a score of recognized species have been found widely distributed in a variety of natural and processed foods, it is obvious that a number of species must have frequent access to the mouth. It seems unlikely that many of these could become adapted to existence in the oral cavity, but it may be assumed that some of them can persist for a time at least in food masses trapped between the teeth, in occlusal crevices, and about the gingival margins. Thus, cultures as usually prepared from saliva and other material from the mouth might be expected to contain more than one variety or species of lactobacillus.

The presence of lactobacilli in intimate relationship with one or more stages of the carious lesion is recognized by most investigators in this field, and a considerable body of evidence supports the belief that these microorganisms are associated with dental caries. Regardless of the nature of this association (whether causal, merely secondary or even accidental) and entirely apart from the possible or probable roles of the other microorganisms in the disease, it is desirable to determine whether more than one species, a single species, or perhaps only certain types of one species are involved. Satisfactory solution of this question necessarily depends upon the use of characteristics with which the limits of the groups under study can be sharply defined. In the past differentiation has been difficult because of the wide variation in morphology and biochemical activities of these organisms (4a) and because of unsatisfactory results of such immunological reactions as have been applied, namely, the agglutination test (2, 5, 6) and the complement fixation reaction (7, 8). The difficulties encountered are probably due, in part, to the presence in different lactobacilli of common antigenic constituents which in these tests may mask the specific reactions

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of more narrowly defined groups. It is now well known that microorganisms, which in many respects are apparently identical, may be differentiated by means of the immunological reactions of isolated chemical constituents of the cells. Classical examples are the types of pneumococci and Friedländer bacilli which are distinguished by differing immunological reactions of the polysaccharides contained in the capsules surrounding the cells. Similar sub-groupings have been intensively studied in other bacterial species including *Streptococcus hemolyticus* (9a, b), *Streptococcus viridans* (9c), and staphylococci (10a, b), all of which are represented in the bacterial flora of the mouth.

The existence of a type specificity among cultures of lactobacilli isolated from the mouth has been demonstrated (4b). Using the method of test tube precipitation, it was shown that bacterial carbohydrates extracted from these organisms react with antisera from rabbits immunized with culture vaccines. By means of these reactions, strains of lactobacilli isolated from the mouth were classified into 2 groups: one containing four or more immunological types, the other composed of immunologically heterogeneous strains. A study of hypersensitivity reactions was planned as a further application of immunological methods to the investigation of the interrelationships of oral lactobacilli. The usefulness of skin reactions in the study of the capsular polysaccharides of the pneumococcus, as well as for the differentiation of types within the species, was clearly demonstrated by Francis and Tillett (11). It is now well known that the reactions are similarly applicable to many bacterial species.

This preliminary communication on the subject of skin reactions to oral lactobacilli is concerned with the production and demonstration in rabbits of a state of hypersensitivity to these organisms.

MATERIALS AND METHODS

Antigens. Cultures were incubated in Jay's broth (12) for 3 days at 37° C. The cells were collected by centrifugation, washed 3 times in a sterile 0.85 per cent salt solution and suspended to the original volume in saline. The suspensions were heated to 60° C for 1 hour, tested for sterility and then stored in the refrigerator until used. A sufficient quantity of vaccine was prepared at one time to complete the whole course of injections in a given experiment. Five strains of oral lactobacilli were used, Numbers 6, 16, 29, 39 and 46, isolated from salivary samples of patients in the dental clinics. Strains 6, 16, 29 and 39, morphologically similar to Hadley's Group I, were isolated in July, 1937. Strain 46 is morphologically similar to her Group II and was isolated in September, 1937. All of them were typed by serum precipitation tests before these experiments were begun.

Sensitization. Two rabbits were used for each vaccine. Each animal received 0.1 cc. of the vaccine intradermally on the abdomen in sites about 1 inch apart once weekly for a period of 7 weeks. The injection sites were examined in 24 hours, again in 48 hours, and then at 2 day intervals as long as any reaction remained. The lesions were measured in length and breadth in millimeters and examined for the presence of erythema and hyperemia, the presence and elevation of edema, and occurrence of induration, necrosis and ulceration.

Carbohydrate extracts. Purified bacterial carbohydrates were prepared by a method previously described (4b). The organisms were collected from 6 to 8 liters of broth culture and resuspended to form a thin paste in 15 cc. to 20 cc. of sixteenth normal hydrochloric acid. This suspension was heated in boiling water for 10 minutes and then chilled quickly and centrifuged. After centrifugation the supernatant extract was decanted and purified by repeated precipitation with 95 per cent ethyl alcohol from clear water solution. Before use in the hypersensitivity tests the carbohydrates were tested *in vitro* for reactivity with previously prepared immune sera. Only those preparations which reacted strongly in high dilution were utilized for skin testing. The reactions of the 5 carbohydrates in their respective bacterial antisera are shown in Table I.

Skin and ophthalmic tests. The presence of hypersensitivity in the vaccine sensitized rabbits was tested by skin injections of purified bacterial carbohydrates extracted from the same cultures. The carbohydrate solutions were prepared from dry powdered extract. Carbohydrate solutions, 0.2 per cent,

TABLE I

Precipitation reactions of carbohydrates extracted from five strains of oral lactobacilli in homologous antibacterial serum

SERUM ANTI:	DILUTIONS OF CARBOHYDRATE, EXPRESSED IN THOUSANDS					
	1:1	1:5	1:10	1:50	1:100	1:500
39A	++++	++++	++++	++++	++++	+++
6A	++++	++++	++++	++++	+++	++
16A	++++	++++	+++	++++	+++	±
29A	±	±	++	+++	++++	++++
46A	++++	++++	++++	++++	+++	+++

++++ Heavy precipitate, firm disc or large flocs; +++ Marked precipitate, usually a disc; ++ Thin disc or small amount of flocculent precipitate; ± Cloudy with no sediment. Serum and carbohydrate controls were negative in each test.

1 per cent and 2 per cent, were made in sterile 0.85 per cent salt solution. These were injected intradermally on the abdomen in 0.1 cc. amounts. The ophthalmic tests were made by injecting approximately 0.05 cc. of carbohydrate solution between the lamellae of the cornea and as near the surface epithelium as possible.

RESULTS

The approximate areas of skin involved in the reactions to vaccine after each weekly injection are presented in *fig. 1.* Minimal and maximal responses are compared with the average responses 24 and 48 hours after each vaccination. *Fig. 2* depicts a comparison of the durations of these lesions.

After disappearance of the inoculation bleb, within 30 to 45 minutes, no perceptible reaction to the primary intradermal vaccine injection occurred within the succeeding 12 hours. At 24 hours an elevated, erythematous, nodular lesion, usually measuring 4 by 5 mm., was present at the site of injection. Within 48 hours, the lesion increased in size to about 7 by 8 mm. After that time it

gradually diminished in size and without necrosis or ulceration disappeared within 6 to 9 days leaving no scar. There was some variation in the reaction among the different animals, but in general the lesions were remarkably constant. Secondary reactions have been observed at the site of the healed initial lesions produced by intradermal inoculation of pneumococci (10c), staphylococci (10d)

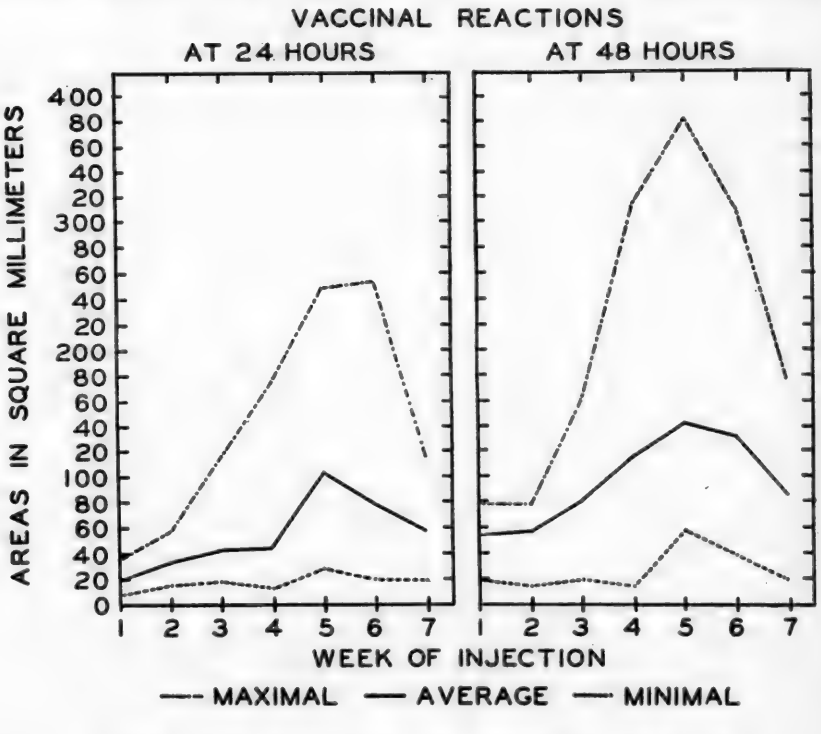

FIG. 1. Approximate areas of minimal, maximal and average lesions 24 and 48 hours after injection of 0.1 cc. of oral lactobacillus vaccines once weekly for a period of 7 weeks. Since the lesions were nearly circular the figure was constructed from computations obtained from the average diameters of the vaccinal reactions.

and *Streptococcus viridans* (13). No secondary reactions were seen in any of the animals used in these experiments.

Although the initial reaction had diminished, all traces had not disappeared by the time the second injection was made 1 week later. The reaction to the second injection seemed to arise more quickly than the first, usually appearing within 18 hours and lasting 6 to 7 days. As may be seen in *figs. 1 and 2*, the intensity and duration of the subsequent reactions continued to increase with repeated injections until they reached their maxima between the fourth and sixth weeks. In

every case these skin reactions to vaccine were much more severe than earlier reactions, being 2 to 4 times as large and persisting 3 to 6 times as long.

The maximal lesions observed at the peak of the reactions were elevated areas of erythema and hyperemia, with induration about 1½ cm. in diameter, 48 hours

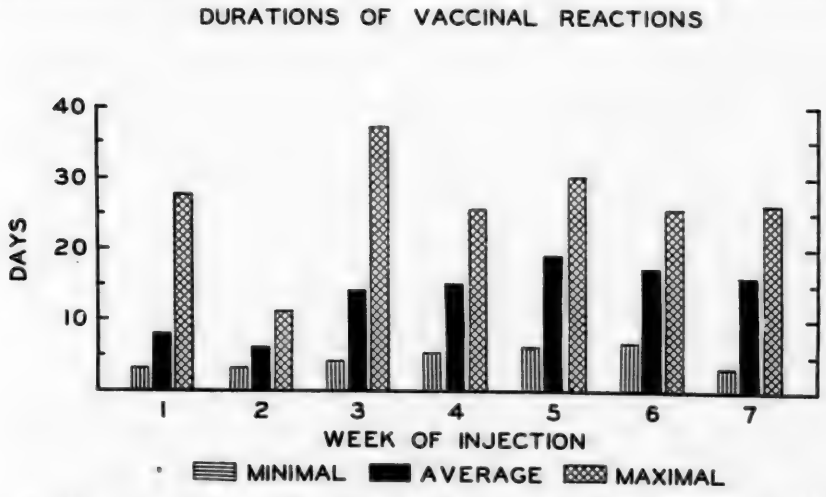

FIG. 2. Minimal, maximal and average durations of reactions after injection of 0.1 cc. of oral lactobacillus vaccines once weekly for a period of 7 weeks.

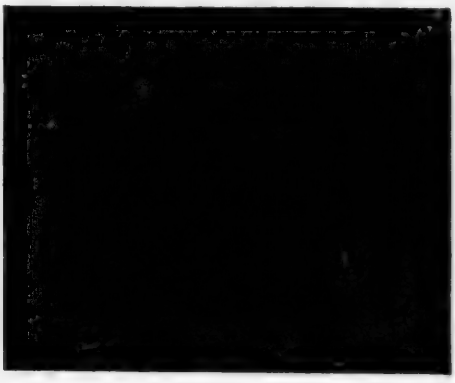

FIG. 3. Illustrates a typical vaccinal reaction photographed at the time of maximal response. Note the cone like elevation and the surrounding area of darkened (erythematous) skin. The limits of the hyperemic area (176 square millimeters) were outlined on the skin with ink.

after injection. *Fig. 3* illustrates a typical vaccinal skin reaction photographed at the time of maximal response. In contrast with the reactions to first injections which persisted a little over a week, several weeks elapsed before complete healing occurred. There were no residual scars, although some of the lesions just

described developed necrotic centers and later ulcerated with a discharge of purulent material. After ulceration these lesions usually retrogressed more rapidly than those not showing ulceration. There was a diminution in the intensity and duration of the reactions if weekly intradermal injections were continued after the maximal response had been attained, the later reactions, except for central necrosis, being much the same as those in the first and second weeks.

Since the increasing reactivity of the skin, indicating increasing hypersensitivity, reached its peak at around the fifth injection of vaccine, this time was chosen for skin tests with the carbohydrate fraction. All rabbits received an intradermal injection of 0.1 cc. of a 0.2 per cent solution of purified bacterial carbohydrate just prior to the fifth inoculation of vaccine. A positive reaction appeared in each animal. The responses were of the delayed type, not becoming definitely perceptible until about 24 hours after injection and not reaching their maxima until the second day. In 48 hours the reactions were flat, hot, erythematous areas usually measuring approximately 18 by 18 mm. in diameter. There was little subsequent spreading, and after another 36 to 72 hours the erythema faded and the lesions became pale, elevated, edematous nodules about 9 by 9 mm. in diameter and elevated approximately 1 to 2 mm. at the center. They persisted for about 6 days and then disappeared without intervening ulceration or necrosis. In addition to the tests with carbohydrates prepared from the same cultures as the vaccines, each sensitized animal was tested also with heterologous carbohydrates from 1 or more other cultures. In most cases the reactions were entirely similar though usually much less marked. Since this evidence of cross reactivity does not coincide with the results of *in vitro* tests, further comparative investigations are being made.

Since it was observed that the reactions to vaccine began to diminish in intensity after the sixth week, it was of interest to determine whether the skin sensitivity to carbohydrate also was decreased, and therefore tests were made again 3 weeks after the seventh vaccine injection. The reactions elicited were less intense and somewhat smaller than those described above and, therefore, may be interpreted as indications of a declining though still persistent hypersensitivity.

The skin responses produced by the injection of 0.2 per cent carbohydrates indicate hypersensitive responses but do not entirely exclude the possibility of toxicity of the carbohydrate. Control tests on toxicity of the purified carbohydrate preparation were made before use in tests on sensitized animals. Single intradermal injections of 0.1 cc. of 0.2 per cent, 1 per cent and 2 per cent solutions into an unsensitized rabbit resulted in no perceptible response. Two unsensitized rabbits were inoculated intradermally once weekly for 7 weeks with 0.1 cc. of a 0.2 per cent carbohydrate solution. At no time was there a response to the injected solution, indicating that at least in these dilutions the extract is not toxic for rabbits and does not elicit skin reactivity.

Since the more concentrated solutions of carbohydrate failed to elicit toxic reactions in unsensitized rabbits, plans were made to observe the effect of these solutions in skin tests on sensitized animals and to compare the results with tests

made with less concentrated solutions. New animals were sensitized by the same technique as described above, and skin tests were performed with 0.2 per cent carbohydrate as well as carbohydrate solutions 5 and 10 times as concentrated (1 per cent and 2 per cent). The resulting reactions to 1 per cent and 2 per cent were of the same delayed type as described above, but they were much more marked. The maximal reactions consisted of elevated, erythematous, nodular areas measuring about 3 cm. in diameter 48 hours after injection. This represented a total area of 705 mm.^{2} as compared with the relatively flat lesions of 55 mm.^{2} which resulted from test doses $\frac{1}{10}$ this concentration. Not only were these hypersensitive responses larger in area of reaction, but the lesions were more severe. The initial, severe inflammation was followed after 3 to 4 days by ecchymosis and then a scaly desquamation. The skin reactions did not persist much longer than those produced by the more dilute carbohydrates, however, and had completely disappeared in 7 to 10 days.

In *fig. 4* the sizes of successive vaccinal lesions in one animal and its hypersensitive skin response to carbohydrate are compared, and the increasing size and duration of the vaccinal lesions are shown. All were drawn to scale. The central outlines shown in the fifth and sixth vaccinal reactions delimit the sharply defined nodules which were present in these 2 lesions.

Ophthalmic tests were performed on 6 rabbits during the peak of skin reactivity by an intracorneal injection of about 0.05 cc. of a 0.2 per cent homologous carbohydrate solution. Spreading of the corneal lamellae, produced by injection pressure, resulted in a small opaque white bleb which slowly subsided without inflammation. No perceptible reaction to the carbohydrate appeared during the succeeding 3 days. On the fourth day the conjunctiva became hyperemic with injection of blood vessels, and the cornea developed an opalescent cloudiness which spread slowly from the site of injection. The maximal intensity of these changes was observed on the sixth day. Following closely after the beginning of these reactions and developing along with them was a corneal vascularization, or pannus, such as that described by Julianelle (10c), typical though not severe. This began as small capillary loops extending into the cornea from the margin of the sclera. The small loops subsequently branched into twig-like vessels which at the time of greatest development projected about 2 mm. into the cornea and had the appearance of a fine, thick brush extending all the way around the cornea. The pannus persisted longer than the conjunctival inflammation, but in about 10 days there was complete retrogression of the capillaries with the return of the cornea to its normal avascular state. That this reaction was referable to the hypersensitive state of the rabbit and not to toxicity of the carbohydrate solution was shown by the absence of any reaction when the same injection was made into the eyes of 3 unsensitized animals.

Sera obtained from all rabbits before sensitization and immediately prior to the skin tests were examined for the presence of agglutinins and carbohydrate precipitins. Negative results were obtained in all of the precipitation tests. This apparent indication that precipitating antibodies are absent from the serum seems to be in accord with the findings of other workers who have employed

intradermal injections of heat-killed organisms in the production of a hypersensitive state (10c, 14). Agglutinins were found, however, in all the sera although they were not present in sera obtained before vaccination. The results of agglutination tests are recorded in Table II. Titres of 1:80 to 1:160 were the

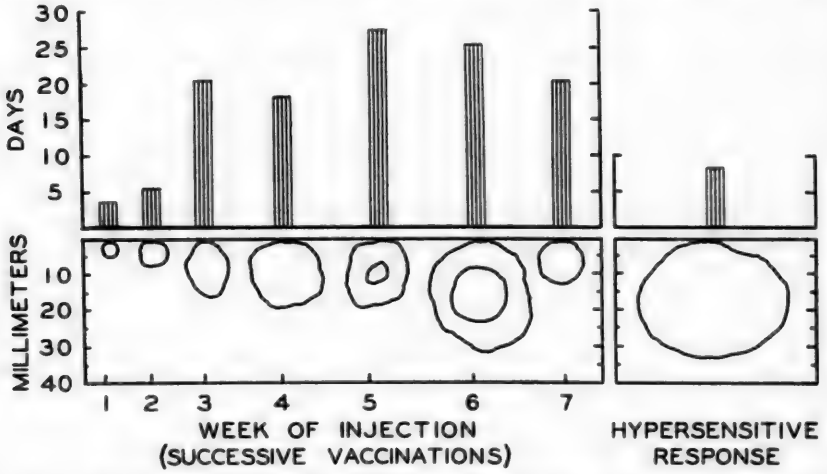

FIG. 4. Sizes and durations of skin reactions to oral lactobacillus vaccine and hypersensitive skin response to 2 per cent purified bacterial carbohydrate injected during the fifth week. All are from 1 animal and are drawn to scale. The central outlines in the fifth and sixth vaccinal reactions delimit sharply defined nodules which were present in these 2 skin lesions.

TABLE II

Agglutination reactions of sera from sensitized rabbits bled 3 weeks after final vaccination

SERUM ANTI:	ANTIGEN	FINAL DILUTION OF SERA						
		1:20	1:40	1:80	1:160	1:320	1:640	1:1280
39	39	++++	++++	++++	+++	+++	++	0
39	39	++++	++++	++++	+++	+	±	0
16	16	++++	++++	+++	++	0	0	0
16	16	+++	+++	++	+	0	0	0
29	29	++++	++++	++++	++++	++++	+++	+
29	29	++++	++++	++++	++++	+++	+++	±

All control tests were negative.

Normal sera from the same animals prior to sensitization were negative in parallel agglutination tests.

most common range, but a few sera showed titres of 1:320 and 1:640. The sera of other similarly sensitized animals possessed agglutinins in the same range.

DISCUSSION

A progressive increase in the intensity of skin reactions to successive injections of vaccine served as a good indicator of the development of the sensitized state.

The greatest skin reactivity to bacterial carbohydrate was reached after 4 to 6 intradermal inoculations of vaccine had been made. A diminution in intensity and duration of reaction to subsequent vaccine injections, indicating an increasing immunity, apparently accompanied a decreasing skin sensitivity.

Although the reactions to purified carbohydrate solutions indicate a sensitivity to these fractions, so far they have not proven to be type specific. Cross reactions were obtained with carbohydrates which showed no cross reactions in precipitation tests. Even though the carbohydrate solutions themselves did not appear to be antigenic and therefore may be assumed not to have been contaminated with protein material, it is possible that small amounts of protein were present. The cross reactions would be explained if minimal amounts of protein, sufficient to elicit a reaction in the skin, could be shown to be insufficient to stimulate antibody production or to form precipitates in the test tube. It is also possible that, in addition to the type-specific carbohydrate, the solutions contain a second carbohydrate or other nonantigenic substance which is reactive in the skin of sensitized rabbits but not in the test tube; and also possible that the carbohydrates that are type specific in precipitation tests possess common radicals which are responsible for skin reactivity. Experiments to test these possibilities have been devised.

The inflammation and vascularization obtained in the ophthalmic tests were similar to conjunctival and corneal reactions previously reported in animals sensitized with any one of the following: egg albumin, bacterial nucleoprotein (10e), solutions of pneumococci which have had acid precipitable and heat coagulable proteins removed (10c). Skin reactivity as well as eye reactivity to higher dilutions (1:1000, 1:10,000, 1:25,000) of type specific carbohydrates of pneumococci (10c) and staphylococci (10d) in similarly sensitized animals have been reported as negative. Most of the tests with the lactobacillus carbohydrate solutions, all giving positive reactions, were made with 1:50 to 1:1000 dilutions. However, definite reactions in the skin of sensitized animals have been obtained with dilutions as high as 1:50,000.

The absence of precipitating antibodies in the sera of animals which have received repeated intradermal injections of heat-killed organisms has been a commonly reported finding and seemed to be indicated by the results of tests with the lactobacilli. However, the fact that no precipitins could be demonstrated in these sera by the *in vitro* reaction does not exclude the possibility that such antibodies may be in the serum in too small amounts to be detectable by this test. The positive response in the skin, which is interpreted to be the result of a reaction between carbohydrate and antibody, indicates that this is a more sensitive test. Precipitins might be demonstrated *in vitro* by a more sensitive method. This has been shown to be the case in the sera of animals hypersensitive to crystalline egg albumin by Cannon and Marshall (15) through the use of a collodion agglutination method. Preliminary tests on lactobacillus carbohydrates with this method have shown that precipitins are present here also in low titre.

One of the goals of the investigation was the differentiation of the types or groups which might be useful in the study of the relation of this microorganism

to dental caries. This has not yet been realized since the tests have not conclusively distinguished between the various strains of oral lactobacilli tested up to this time. There were certain suggestive group differences, however, and it is not unlikely that further study, refinement of technique and use of additional strains, will result in the establishment of definite group relationships. The investigation is being continued.

SUMMARY

1. The repeated injection of heat-killed lactobacilli of human oral origin into the skin of rabbits elicits a hypersensitivity which can be demonstrated by intradermal and intracorneal injection of nontoxic cellular carbohydrate extracted from these organisms. The skin reaction is of the delayed type.

2. The reactivity increased progressively until 4 to 6 successive intradermal inoculations of vaccine had been made and then diminished in intensity and duration.

3. Type specific precipitins were not demonstrable in the sera of the sensitized animals by ordinary *in vitro* precipitation tests, but lactobacilli were usually agglutinated by these sera in low titre.

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DENTAL STUDIES OF TRIPLETS

I. Measurements of Arch Width and Length[1]

J. T. COHEN, D.D.S., C. P. OLIVER, Ph.D., and S. BERNICK, D.D.S., M.S.

The members of the dental profession, particularly orthodontists, are interested in the effects of heredity and environment in the development of occlusion. It is recognized that both environment and heredity are active in the development of a character. Twins or triplets of monovular origin develop from a single fertilized ovum, and have identical hereditary factors; therefore, differences between two individuals of the set may be considered due to environmental influences. Non-identical individuals of a set develop from separate ova, and have hereditary factors no more alike than any set of siblings; but they have developed under similar conditions. The differences and similarities observed in a fraternal set can be used in a comparative manner in studies of identical individuals.

Several investigators have used identical twins in their studies of the relative importance of heredity and environment in the development of dental arches. Goldberg (1929) concluded that, except in individual cases where abnormal habits have interferred with intrinsic forces, hereditary factors determine the arch form and that environment, either intra-oral or extra-oral, under average conditions has little part in the determination of the arches. Bachrach and Young (1927) reported that the concordance of normal occlusion is significantly higher in identical pairs than in fraternal twins, and that identical twins present a closer coincidence in the type of malocclusion than do fraternal twins of like sex or unlike sex. Korkhaus (1930) recognized differences and anomalies which were due to extrinsic factors, but he observed that the identical sets were relatively similar in their dental conditions. Braun (1938) concluded that heredity has the most important part in the determination of the size and shape of the dental arch. The arches of fraternal twins were found by Braun to be less alike than those of identical twins, but more alike than those unrelated pairs re-arranged at random from the twin sets, indicating that a partially common heredity was influential.

Newton (1937), in a report on a set of triplets, found that the arches of two members of the set were alike but the third member's were dissimilar. With the recent interest of the orthodontist and geneticist in the effects of heredity

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and environment on the arches, all information on the subject material should be gathered. For that reason, this study of the dental arches of 9 sets of triplets is reported.

MATERIALS AND METHODS

The subjects for this study were selected from a group of children who were studied at the Institute of Child Welfare of the University of Minnesota. Impressions were taken of the dental arches of 9 sets of the triplets whose ages ranged from 4 to 16 years. Study casts were poured in plaster and measured. The same technique for measurements were employed as those used by Cohen (1940).

The 9 sets of triplets have been studied by Rigler (1938) who reported on the skeletal similarities as shown by means of x-ray studies, and by Howard (1934) who used 7 criteria in the determination of the identical and fraternal members of the sets. In this study, we are primarily interested in the possible use of arch size as shown by measurements as a means to determine the monovular origin of twins. Consequently the conclusions on monovular origin were made with the measurements as bases and were checked against the conclusions made by Howard and Rigler.

Some of the observed similarities between the arches of identical twins were due to the identical genetic background of the individuals, but chance occurrence in subjects of the same age and sex may also be a factor. Braun (1938) used control subjects with his twin studies. He selected his controls by rearranging the twins to constitute unrelated pairs, a method which has some chances for errors. We were able to use as control subjects non-related children who had been at the Institute of Child Welfare. Three members were chosen at random from a given age and sex group. Three groups were selected as control sets. In the control sets, C2, corresponds to triplet set II; C3 to set III; and C7 to set VII. The measurements for the control individuals had been made by the same operators who studied the triplet sets. Each of the 3 control sets can be used for comparative purposes only with the triplet set of that age and sex. The comparative measurements actually measure only the degree of similarity as a result of a given age and sex.

RESULTS

For convenience in reference, each triplet set is represented in the data by a Roman numeral, and the control set by an Arabic numeral. The symbols X, Y, and Z refer to the respective members of a set.

The measurements shown in the table represent the averages of independent measurements which were made by 3 operators. Consequently, it becomes necessary for the purpose of interpretation to correct for possible deviations from the true measurements, and to determine how much difference between measurements can be allowed while accepting the measurements as identical. The measurements made by the 3 operators on any one individual varied from 0 to 1 mm.; occasionally the variation was greater than 1 mm. The average varia-

tion approached 0.5 mm., and that value was taken for the determination of "corrected identical measurements." Arbitrarily, we set the standard that if 2 of the average measurements came within 0.5 mm. of each other they should be considered to be identical.

I. The first set of triplets to be considered were 3 girls, who were 4 years of age. In the canine to canine relationships as well as in the length measurements, members Y and Z were similar. The measurements of X were in general the widest and longest in both arches. If allowance is made for the variation in measurements due to the individual differences by the operators ("corrected identical measurements") members Y and Z were identical for 3 of 9 measurements. The low concordance indicates that Y and Z were not monovular in origin. Although Y and Z were found to be very similar in skeletal growth (Rigler) and in most of the classifications used by Howard, they were not considered to be identical twins since they differed in their blood grouping.

II. The second set included 3 girls, 6 years of age. In the dental measurements, no 2 individuals were identical. Member Y was, in general, intermediate between X and Z

TABLE I

Proportion of identical measurements

SET	SEX	AGE	NUMBER OF MEASUREMENTS	PER CENT IDENTICAL MEASUREMENTS		
				XY	XZ	YZ
I	♀	4	9	22.2	11.1	33.3
II	♀	6	14	35.7	14.3	28.6
III	♂	6	14	21.4	64.3	21.4
IV	m	7	11	27.2	45.4	18.2
V	♀	9	19	68.4	26.3	36.8
VI	♀	9	8	0.0	50.0	12.5
VII	m	13	13	23.0	30.8	7.7
VIII	m	14	9	33.3	22.2	33.3
IX	♀	16	25	28.0	28.0	44.0
C2	♀	6	14	28.6	35.7	28.6
C3	♂	6	14	35.7	21.4	50.0
C7	m	14	20	35.0	25.0	15.0

for both the cusp to cusp and the length measurements. Using the corrected measurements, one finds that Y was similar to X and to Z with approximately equal frequency. Members X and Y were identical in 5 of the 14 measurements; Y and Z, in 4 measurements. On the basis of the dental measurements, the set should be considered to be fraternal (trizygotic) triplets, a conclusion supported by the control set for this group (see C2). However X and Y were considered on the basis of skeletal studies (Rigler) and by the criteria of Howard to be identical twins.

III. The third set studied was made up of males, 6 years of age. A comparison of the dental measurements indicates that X and Z had a high frequency of similarity in the measurements for the widths and lengths of the arches. On the basis of allowed variations, X and Z were identical in 9 of the 14 measurements. The concordance of X and Z was high, and they can be considered to be monovular in origin; Y developing from a second ovum. The members X and Z were considered to be identical twins by Howard and Rigler.

IV. The fourth set examined included 2 boys and a girl 7 years of age. Since this set included both sexes, the interest for possible monovularity was limited to the 2 boy members Y and Z. The measurements of the 2 boys were definitely unlike, except for occasional similarities which might be expected of the individuals in the same age and sex group.

The arch measurements of the girl, X, resembled those of one of the boys, Z, more frequently than the 2 boys resembled each other. The boys were identical in only 2 of the 11 measurements. Mutilations of the arches were present in the boys, but the measurements differ considerably even in the incisal and molar regions in which no mutilations had occurred. On the basis of the dental measurements, the 2 boys were considered to be dizygotic in origin. That interpretation agrees with the conclusions of Howard and Rigler.

V. The fifth set included 3 females 9 years old. The measurements indicated that all 3 members had relative similar widths and lengths of the arches, although in most measurements Z had the narrowest and shortest arch. In the dental measurements, X and Y had a higher degree of concordance than Z had with either X or Y. On the basis of corrected identical measurements, X and Y were identical in 13 of the 19 measurements, and can be considered to be monovular in origin. Members X and Y were considered to be identical members by Rigler and Howard.

VI. The sixth set consisted of 3 girls 9 years old. This group has been reported in detail by Lund (1933), who considered the members to be identical triplets. The dental material on this set is included in our study only to show the extreme effects of the extrinsic factors on the dental arches. The arches of all 3 girls had been more or less mutilated by the early loss of the deciduous teeth and by caries in the permanent teeth. Member Y, for instance, had lost the right upper second deciduous molar; her upper first right permanent molar was carious and had tipped mesially, thus shortening the arch; and the right side had collapsed in a bucco-lingual direction resulting in a narrowing of the arch. In all of our tests, no 2 members had close likenesses. Members X and Z are identical for 4 of the 8 measurements. On the basis of dental measurements alone, this set would be considered to be trizygotic triplets.

VII. The seventh set of triplets was made up of 2 boys and a girl 13 years of age. The two boys, X and Y, on the basis of the corrected dental measurements were very low in the degree of similarity, being identical in only 3 of the 13 measurements. They differed more than the 2 boys in their control set, C7. Therefore, they would be classified as fraternal; but because of an uncommon anomaly in the 2 boys, we concluded that they were monovular in origin. Each boy had 6-cusp, lower, first permanent molars. The girl member had the normal lower 5-cusp first molars. It would be unusual to find this hereditary rarity in 2 siblings if they were not identical twins. Howard and Rigler concluded from their study that the 2 boys were identical twins.

VIII. Two boys and a girl 14 years of age formed the eighth set. It was impossible to study this case in detail owing to the mutilations of the mouth. For the measurements available, the 2 boys did not show a high degree of concordance. On the basis of corrected variation they were identical in 3 of the 9 measurements, and should be considered trizygotic in origin. This agrees with the conclusions of Howard and Rigler.

IX. Three girls, 16 years of age, were examined in set nine. In the measurements, X tended to have the narrowest arch, although her arch length measurements were relatively similar to those of Y and Z. On the basis of corrected identical measurements, Y and Z were identical in only 11 of the 25 measurements, and therefore not monovular in origin. However, they were more similar than pairs of some of the triplets sets which were considered by Howard and Rigler to be identical. Rigler and Howard also concluded that members Y and Z were fraternal.

C2. The first group of controls, 3 non-related females, 6 years old, should be compared to triplet set II. No 2 members of the control set showed any close degree of similarity in the measurements; and any 2 members were identical with a frequency approximately the same as that of any other 2 members. On the basis of the allowed variation of 0.5 mm., X and Z were identical in 5 of the 14 measurements as against 4 in either of the other member combinations. However X and Z of this set were as identical as were the monovular individuals in triplets set II, for which this is the control. On the basis of dental measurements the control set C2, and the triplets set II are equally variable.

C3. Three boys, 6 years old, were selected as controls for triplets set III. Members Y and Z were more alike than X was like either one; Y and Z were identical in 7 of the 14

measurements, a relatively high degree of concordance for 2 non-related individuals. However, they were more variable than were the 2 identical members of triplet set III, for which they were used as a control set.

C7. The third group, 2 boys and a girl age 14, represented the control study for triplet set VII. In the arch measurements the girl had the widest and longest arch for most measurements; consequently the 2 boys were more alike than either resembled the girl. The 2 boys, however, were identical (allowing 0.5 mm. variation) in only 7 of the 20 measurements. They were more identical than the 2 boys in triplet set VII for which they were a control.

The data collected for the 9 sets of triplets and the 3 sets of controls are summarized in Table I. In column 1 Roman numerals represents the triplet sets; the Arabic numerals, the controls. In the second column the sexes are given, with "m" referring to those sets having 2 boys and 1 girl; in set IV the girl is member X and in sets VII, VIII, and C7 the girl is in each case member Z. Column 4 gives the total number of measurements made. In columns 5, 6 and 7 are given the per cent of identical measurements (as determined by the 0.5 mm. allowance) for each combination of the members of the set. If more than 2 of the combinations were identical for any type of measurement, that measurement was added to each set involved. By the criteria of Howard and of Rigler, identical members are XY in set II, XZ in III, XY in V, XYZ in VI and XY in VII.

DISCUSSION AND CONCLUSIONS

The dental measurements representing the arch size for 9 sets of triplets and 3 sets of non-related individuals were determined. The degree of concordance based upon the "corrected identical measurements" has been determined for each combination of the members of a set.

Of the 9 triplet sets studied here, only sets V and III are found each to have 1 pair of members having a relatively high degree of concordance (68.4 and 64.3 respectively). Sets II, VI and VII are considered by all other criteria each to have 1 pair, monovular in origin, but each pair is no more similar in dental measurements than are pairs in triplet sets of trizygotic origin or pairs in non-related sets. Sets II, with the members having all the teeth present, shows as much variation as does its control set (C2). Under the method of study used here, the measurements for set II show how different identical twins can be. The members of set VI, all 3 identical by all other criteria, differ considerably. No 2 members of this group show a high concordance. Only 2 members of this set (X and Z) show any sort of similarity, and they are identical in only 4 of the 8 "corrected measurements". In part, at least, the discordance in VI is due to severe mutilations of the arches. The 2 boys of set VII are least alike of all the sets studied, including the control of this set. Only because of a dental anomaly in both boys were they regarded as monovular in origin.

The 4 sets considered by other criteria to be fraternal in origin (I, IV, VIII, IX) vary in the degree of concordance. Members of either set I or IX vary in their measurements as might be expected for sisters of the same age and sex. The other 2 fraternal groups are of mixed sex; and the girl member in each case is at least as identical in measurements to 1 boy as the 2 boys are to each other.

In the control groups, members of set C2 show no greater variation than

do those of triplet set II. The pair of individuals most alike in C2 has the same proportion of identical measurements as the pair (identicals) most alike in II. Control set 3 also has a pair more identical in measurements than are the pairs in most of the triplet sets. The pair in C3, however, does vary more than the identical member of set III, the unrelated boys of set C7 are even more similar in their measurements than are the boys of set VII. They are as identical as some of the fraternal triplet sets.

From our studies it seems that likeness in twins is due not only to a common heredity, but also to like age and sex. Any 2 members of the control set 3 are as similar in dental measurements as any 2 related members of the triplet set III other than the identical pair. Sets C2 and II especially show the similarities due to like age. Members of triplet set II with a common genetic background and a common uterine environment are no more alike than the 3 non-related members in the control set of that same age and sex.

At certain age periods one expects to find a different rate of growth occurring in the jaws. Between the ages of 6 and 7, for example, an active growth takes place in the incisor region due to the replacement of the deciduous incisors by the permanent successors. No two individuals (even identical twins, see V) erupt their teeth at the same time. In the comparison of a triplet set of 6 years with a random group composed of subjects of 3 different ages (6–7–8) one should expect a wide variation in the non-related individuals. But if age is controlled, one finds that the non-related pairs show no more variation than do their comparative triplet sets in this study.

Dental measurements as studied here cannot be used alone to determine monovulars in twins and triplets. Identical individuals may be relatively similar in their measurements (sets III and V); but some twins classified as identical by other criteria vary considerably in their dental measurements. Fraternal twins in these studies show as much variation as do non-related pairs of like age and sex. The discrepancies found between the arch measurements and the other criteria may be explained by the regressive effects of certain extrinsic factors. Mutilation and exfoliation of the teeth leading to temporary conditions occur at different times in the members of a set and cause variations in the dental measurements (Cohen, Oliver and Bernick, 1942). These data indicate that caution is needed in the use of dental characteristics for the determination of monovularity. A single study at one time in the life of the individuals, may, because of temporary conditions and diseased conditions, lead to wrong conclusions.

SUMMARY

Measurements of the arches were made for 9 sets of triplets and 3 sets of non-related individuals. The degree of similarity for the 3 possible combinations of each set, as based upon corrected identical measurements, was determined.

Five of the triplet sets have each one pair of identical twins by other criteria. Only 2 of the 5 have relatively identical arch measurements. The other 3 sets are no more identical than fraternal and non-related sets.

It is suggested that in part the likeness shown by a triplet set is due to the like

age and sex; and that part of the unlikeness in identical sets is due to extrinsic factors. These factors make it difficult to use dental measurements as a means of determining monovular origin.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section[1]

January 29, 1942

Compiled by FRANCES KRASNOW, PH.D., *Editor, New York Section*
Guggenheim Dental Clinic, New York, N. Y.

1. THE EFFECT OF VARIOUS FORCES ON THE ALVEOLAR BONE. *Carl Breitner*, M.D., D.D.S., School of Dental and Oral Surgery, Columbia University, New York. It has been established by clinical observation as well as by animal experimentation that forces in various directions are not equally effective on the alveolar bone. From experimental material already published, as well as from new investigations, a relationship between the degree and manner of action of a certain force has been established. This can be demonstrated in histologic material obtained from animal experimentation.

2. THE UTILIZATION OF LACTIC AND PYRUVIC ACIDS BY ORAL MICROORGANISMS.[2] *Isaac Neuwirth*, Ph.D., and *William H. Summerson*, Ph.D.,[3] Department of Pharmacology and Therapeutics, College of Dentistry, New York University and Department of Biochemistry, Cornell University Medical College, New York. In work previously reported (Summerson, W. H., and Neuwirth, I., *J. D. Res.*, **20**: 157, 1941), it was shown by chemical and manometric methods of analysis that oral microorganisms acting on glucose in saliva as a medium produce significantly more acid than can be accounted for by the amounts of lactic acid and pyruvic acid found. A partial explanation for this is indicated by the work now reported, which shows that oral microorganisms can metabolize both lactic and pyruvic acids almost as rapidly as they are formed. The metabolism of lactic acid leads to an intermediate production of significant amounts of pyruvic acid, but the further metabolism of pyruvic acid produces no lactic acid. Thus the appearance of lactic and pyruvic acids in the metabolism of glucose by oral microorganisms represents an intermediate and not a final stage in acid production. The nature of the acid or acids ultimately obtained is under investigation. These experiments were carried out with saliva obtained from both carious and non-carious subjects. As in our previous work, no significant differences were evident between these 2 groups of subjects with regard to either acid production from glucose or the utilization of lactic and pyruvic acids.

3. RELATIVE ALVEOLOCLATIC EXPERIENCE OF THE VARIOUS TEETH. *Samuel Charles Miller*, D.D.S., and *Benjamin B. Seidler*, A.B., D.D.S.,[3] College of

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[3] Guest.

Dentistry, New York University, New York. This investigation was carried out by recording the relative amount of bone loss around each tooth seen in the radiograms of patients treated in the Periodontia Clinic. Five hundred cases, selected at random, were studied. Their ages ranged from 14 to 70 years; the mean was 39.8 years. Each tooth was given an alveoloclasia number between 0 and 5, depending on the relative amount of bone loss present. The average periodontal indices (sum of the alveoloclasia numbers divided by the number of such remaining teeth) of the various teeth were found to fall into 4 broad categories of relative susceptibility: 1. *Least susceptible:* maxillary and mandibular canines and first and second premolars, mandibular third molars. 2. *Mildly susceptible:* Mandibular first and second molars. 3. *Moderately susceptible:* Maxillary first and second incisors, third molars and the left first and second molars. 4. *Markedly susceptible:* Maxillary right first and second molars and mandibular first and second lateral incisors. Subdivision of age and sex categories was carried out.

4. CULTIVATION OF SPIROCHETES OF THE MOUTH. *Theodor Rosebury*, D.D.S., and *Genevieve Foley*, M.A., Department of Bacteriology, Medical and Dental Schools, Columbia University, New York. To be presented at the 20th General Meeting of the International Association for Dental Research. See proceedings of last meeting to be published in this volume (21).